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FACTORS AFFECTING COMPETITIVE SAPROPHYTIC COLONIZATION OF THE AGAR PLATE BY VARIOUS ROOT-INFECTING FUNGI

By R. L. WASTIE

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(With 2 Text-figures)

Growth rates of fourteen root-infecting fungi in the presence of competing soil micro-organisms were measured on Cellophane film overlying plates of Czapek-Dox + 0·05 % yeast-extract agar, which were inoculated with unsterilized soil at 0–48 hr. before inoculation of the overlying Cellophane with the fungus under test. The results obtained were not significantly affected either by the pH of the agar or by the incorporation of 0·005 % streptomycin to check growth of bacteria. Results reported by Rao (1959) in these *Transactions*, using an agar plate modification of the 'Cambridge method' for estimating competitive saprophytic colonization, were confirmed and extended, particularly with respect to the effect of age of the pure-culture inocula employed for making up inoculum/soil mixtures.

Integration of results obtained by the Cellophane technique and by Rao's method suggests that the success of a fungus in colonizing the agar plate from a mixed inoculum can be predicted simply from a measurement of its linear growth rate in pure culture. No correlation was found between success in saprophytic colonization and tolerance of a fungus to antibiotic effects. It is therefore concluded that the outcome of the struggle for colonization of the agar plate is largely decided by the relative linear growth rates of the competitors before significant antibiotic effects are produced.

As Garrett (1956) has explained, the outcome of any struggle between one particular micro-organism and others for the colonization of a substrate will depend upon two characteristics of the micro-organism concerned, (*a*) its competitive saprophytic ability, and (*b*) its inoculum potential. Garrett (1950) had earlier suggested three factors that are likely to influence the former attribute—growth rate, production of antibiotic toxins, and tolerance of such toxins produced by other micro-organisms. A third important group of factors concerned in saprophytic colonization is constituted by environmental conditions, including in particular the number and variety of competitors in the immediate vicinity of both the particular micro-organism under investigation and the substrate it is about to colonize.

The evolution of a precise technique for estimating competitive saprophytic colonization, bearing in mind the importance of the above three observations, came with the work of Butler (1953*a*), and subsequently that of Lucas (1955) and Macer (1955). Butler and Lucas developed the 'Cambridge method' for such estimations, using four cereal root-rot fungi.

As already quoted, two possible characteristics of a successful competitive

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saprophyte are the production of antibiotic toxins and a tolerance of the toxins produced by its competitors. Dobbs & Hinson (1953) have given evidence of the widespread occurrence in soils of a fungistatic factor inhibiting spore germination, and Butler (1953*b*) showed *Helminthosporium sativum* to be considerably more sensitive to fungal antibiotics in vitro than was *Curvularia ramosa*, a more vigorous competitive saprophyte.

Thus many factors must be considered as contributing to the degree of competitive saprophytic ability of a root-infecting fungus, and in an attempt to simplify the assessment of such activity use has been made by Rao (1958, 1959) of an agar plate modification of the original Cambridge method. In Rao's technique the test substrate for competitive saprophytic colonization was a plate of acidified Czapek-Dox + yeast-extract agar, and Rao was able to classify into three groups the twelve root-infecting fungi he studied, according to their degree of success in saprophytic colonization of the agar plate from a range of inoculum/soil mixtures. Rao then sought some correlation between saprophytic behaviour and two other fungal characteristics: (1) intrinsic growth rate, as measured in pure culture on the agar plate; (2) tolerance to antifungal antibiotics, as measured against a range of ten purified antibiotics in vitro. He failed to find any simple correlation, and so the present paper reports a further attempt to elucidate those growth characteristics of a fungus that contribute to success in competitive saprophytic colonization of the agar plate, by using an improved technique to estimate tolerance to antifungal antibiotics.

Fungi selected for study

Twelve of the fourteen root-infecting fungi selected for study were subcultured from the original isolates used by Rao (1958, 1959), but the last two in the list below were additional species. The original sources of all the fungi studied are indicated below:

	Isolated from	Supplied by
Curvularia ramosa (Bain.) Boedijn	Wheat	Dr F. C. Butler
Fomes annosus (Fr.) Cooke	Pine	Dr J. Rishbeth
Fusarium avenaceum (Fr.) Sacc.	Potato	Dr R. K. McKee
F. caeruleum (Lib.) Sacc.	Potato	Dr M. J. Carlile
F. culmorum (W. G. Sm.) Sacc.	Wheat	Dr S. D. Garrett
F. oxysporum Fr. f. *cubense* (E. F. Smith) Snyder & Hansen	Banana	Dr J. Rishbeth
F. oxysporum f. *vasinfectum* (Atk.) Snyder & Hansen	Cotton	Prof. T. S. Sadasivan
Helminthosporium sativum Pammel, King & Bakke (isolate 1 of Rao, 1959)	Wheat	Dr F. C. Butler
Macrophomina phaseoli (Maubl.) Ashby	Cotton	Prof. T. S. Sadasivan
Ophiobolus graminis (Sacc.) Sacc.	Wheat	Dr S. D. Garrett
Rhizoctonia solani Kühn	Swede	Dr R. K. S. Wood
Verticillium dahliae Kleb.	Cotton	Dr G. M. Wickens
Pythium mamillatum Meurs	Turnip	Dr D. Park
Helicobasidium purpureum Pat.	Sugar beet	Dr T. F. Hering

Effect of other soil micro-organisms on growth rates of the test fungi

In his attempt to discover if a relationship existed between fungal growth rate under conditions of competition and success in saprophytic colonization, Rao (1958) did not make a direct measurement of growth rate under such conditions, but tested each fungus under investigation against a range of ten purified antibiotics. His inability to derive any formula that would correlate success in competitive colonization with growth rate and tolerance to antibiotics for all the fungi tested may have been due to the selection of an unrepresentative range of purified antibiotics, and one way of overcoming this difficulty would be to test growth rates in the presence of the antibiotics formed by competing soil micro-organisms on the acidified Czapek-Dox + yeast-extract agar plates used for saprophytic colonization experiments.

The technique adopted for this purpose consisted in growing each test fungus over a thin sheet of Cellophane film laid on the surface of an agar plate that had been previously inoculated with soil. The soil was placed on the plate as a thin coating at four equidistant loci, and four inoculum disks of the test fungus were placed on the Cellophane so that they exactly covered the soil inocula. The success of the technique depends on the fact that easily diffusible nutrients and fungistatic substances pass quickly through the thin film of Cellophane, but fungal hyphae take some time to penetrate it, and hence the inoculated fungus grows as pure colonies, for some days at least, on the upper surface of the Cellophane. These growing colonies are affected by competition from other micro-organisms growing out from the soil inocula directly beneath them; inasmuch as these other micro-organisms consume the nutrients in solution in the agar gel and excrete fungistatic products, the intensity of the competition can be increased by lengthening the interval between the inoculation of the agar plates with soil and the eventual inoculation of the fungus under investigation onto the Cellophane film, thus allowing the competitors to make a variable amount of growth under the Cellophane in advance of the second inoculation.

In the experiments of Rao (1958, 1959), competition for colonization of the agar plate from the soil component of the mixed inocula which he used was largely restricted to soil fungi, through the deliberate acidification of the Czapek-Dox + yeast-extract agar to pH 4·0. It seemed possible that this degree of acidification with phosphoric acid, necessary to inhibit excessive growth of bacteria, might itself have affected differentially the competitive performances of the various test fungi, and have been the chief factor (rather than competition) depressing colonization by some of the weaker competitive saprophytes. The Cellophane film method has also been used, therefore, to test this possibility, by comparing the growth of the various fungi at pH 6·5 (with streptomycin added as the bacteriostatic agent) with that at pH 4·0. Furthermore, growth rates have been compared at pH 6·5 in the presence and absence of streptomycin, to determine whether the increased growth of soil bacteria in its absence would appreciably augment the competitive effects exercised by the soil fungi alone.

The Cellophane film technique

Preparation of the agar plates. The substrate for competitive colonization was the modified Czapek-Dox agar as used by Warcup (1950), but with the 75 % reduction in the proportion of nitrogen as employed by Rao

(1959) to facilitate recognition of *Fusarium* spp. It had the following composition: $NaNO_3$, 0·5 g.; KH_2PO_4, 1 g.; KCl, 1 g.; $MgSO_4 . 7H_2O$, 0·5 g.; $FeSO_4$, 0·01 g.; sucrose, 30·0 g.; 'Bacto' yeast extract, 0·5 g.; agar, 22·5 g.; distilled water, 1000 ml. The medium was adjusted to the pH required (i.e. to 4·0 with N/20 phosphoric acid or to 6·5 with 4N sodium hydroxide) and autoclaved for 10 min. at 10 lb. pressure. A measured 20 ml. was poured into each 9 cm. Petri dish, and immediately the agar had set the plates were inverted and left for 24 hr. to allow evaporation of the surface film of moisture. This precaution, together with that of acidifying the medium to pH 4·0 or that of adding 0·1 ml. 1% (w/v) aqueous streptomycin to the plate prior to pouring in the agar, was taken to discourage excessive bacterial development after inoculation of the plates with soil.

Preparation of Cellophane disks. A supply of sterilized Cellophane disks, cut from thin (21·6μ) non-lacquered Cellophane film (PT grade 300) to a diameter 0·5 cm. smaller than that of the Petri dishes, was prepared by boiling the disks in distilled water for 15 min. to remove plasticizers. The disks were then autoclaved in fresh distilled water in a deep covered dish for 15 min. at 15 lb. pressure, and kept there under water until required.

Preparation of soil. The soil used in all experiments was the light loam from the Cambridge Botanic Garden, with a pH of 7·2 and a moisture-holding capacity of 50 ml./100 g. air-dry soil. Samples of soil were collected from a field dump, passed through a 2 mm. sieve, and kept moist (at about 40% m.h.c.) until required.

Setting-up the experiments. On the bottom of each Petri dish, four loci were marked out with a glass-marking pencil so that each was approximately at the centre of a quadrant. At each locus, the surface of the agar was then lightly inoculated with soil by gently pressing onto it the end of a stainless steel rod (4 mm. diam.) that had first been allowed to pick up the inoculum of soil by contact; if the end of the rod had previously been made moist by contact with the fresh agar surface, a sufficient amount of soil for a satisfactory inoculum could be picked up. In this way, a light coating of soil over a circle *c.* 4 mm. diam. was deposited on the agar at each locus.

After this inoculation with soil, the surface of the agar on each plate was covered by laying on it a sterilized Cellophane disk (which had been quickly drained of excess water), taking care to avoid trapping air between the surface of the agar and the Cellophane. The Cellophane was next inoculated with one of the various test fungi—either immediately, or after an incubation period that varied according to the particular experimental treatment. The Cellophane was inoculated at each of the four loci with a 4 mm. agar disk cut out with a sterilized no. 1 cork borer from the growing margin of a pilot colony of the test fungus, and so placed that each agar inoculum disk was directly above a soil inoculum, and separated from it by the Cellophane. In each experimental treatment, two plates were inoculated with each test fungus, to give a replication of eight inocula for every fungus-treatment combination. Measurements of colony diameters of the test fungi on the surface of the Cellophane were made every 12 hr. up to 72 hr., using a pair of dividers and a millimetre scale.

Experiment 1. The following comparisons were made: (1) between growth on Czapek-Dox + yeast-extract agar at pH 4·0 and on the same agar at pH 6·5, with the inclusion of 0·005 % streptomycin in the latter plates to replace acidity as the bacteriostatic agent, (2) between plates not inoculated with soil (i.e. sterile plates) and plates inoculated with soil and subsequently incubated for 0, 12, 24, or 48 hr. before the overlying Cellophane was inoculated with the various test fungi. Plates were incubated at 22·5° C. throughout the whole experiment. Table 1 shows the mean colony diameter of the eight replicate colonies of each fungus in every treatment after 59 hr. growth, with three exceptions as follows: for *Pythium mamillatum*, colony diameters are given after 23 hr., for *Macrophomina phaseoli* after 35 hr., and for *Helicobasidium purpureum* after 160 hr. The first two of these fungi had filled the plates by 59 hr., while the third had made only negligible growth by this time.

The fourteen species of fungi for which growth data are given in Table 1

Table 1. *Colony diameters (mm.) of fourteen fungi on Cellophane, overlying plates of Czapek-Dox + yeast-extract agar previously inoculated with soil at various times*

	pH of agar	No soil	Incubation period of soil (hr.)			
			0	12	24	48
*Pythium mamillatum**	4·0	29	31	29	29	9
	6·5	34	33	31	28	14
*Macrophomina phaseoli**	4·0	34	40	39	38	12
	6·5	39	35	38	36	13
Fusarium culmorum	4·0	47	48	47	44	30
	6·5	52	52	50	45	27
F. avenaceum	4·0	34	37	32	29	18
	6·5	35	36	32	27	16
F. oxysporum f. *cubense*	4·0	29	26	23	20	14
	6·5	29	25	24	21	13
Curvularia ramosa	4·0	29	25	24	18	13
	6·5	28	26	26	22	14
Rhizoctonia solani	4·0	27	22	23	22	19
	6·5	27	24	25	23	21
Helminthosporium sativum	4·0	25	26	22	17	8
	6·5	27	25	22	17	9
Fusarium oxysporum f. *vasinfectum*	4·0	23	20	20	23	15
	6·5	26	27	27	26	17
Ophiobolus graminis	4·0	21	17	15	12	6
	6·5	24	20	15	10	5
Fomes annosus	4·0	18	13	8	6	5
	6·5	18	13	7	7	5
Fusarium caeruleum	4·0	17	15	11	8	7
	6·5	18	15	11	10	6
Verticillium dahliae	4·0	13	12	12	8	5
	6·5	13	12	11	10	6
Helicobasidium purpureum†	4·0	18	7	7	5	5
	6·5	15	7	6	5	5

* Growth rates of the first two species listed were higher than indicated by the figures, since colony diameters were measured after a fraction of the standard time.

† Growth rate of *H. purpureum* was relatively lower than indicated by the figures, since colony diameters were measured after 160 hr. instead of after 59 hr.

have been arranged in descending order of growth rates, and considering these rates at the two pH levels it is apparent that the pH value of the agar has had remarkably little effect on the growth rates of the test fungi. The most marked differences are apparent in *Pythium mamillatum*, which grew rather more quickly on the less acid medium. This observation agrees

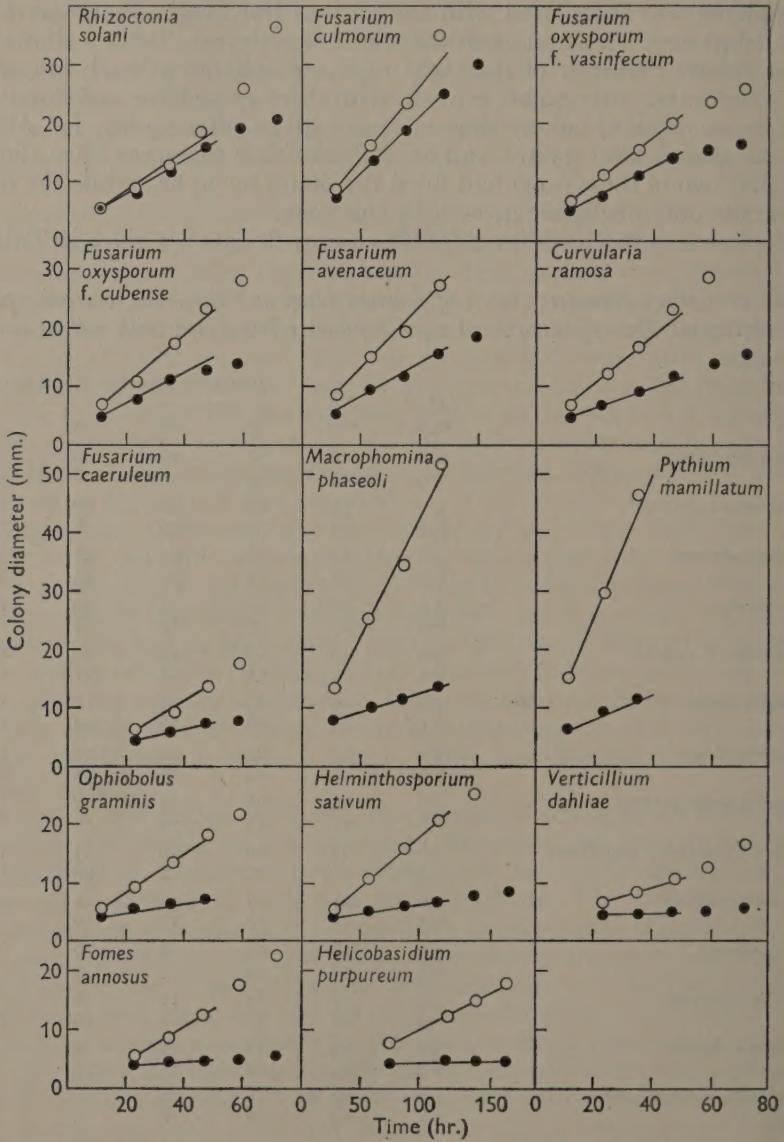

Fig. 1. Growth rates of fourteen fungi on Cellophane, overlying plates of Czapek-Dox + yeast-extract agar, in the presence and absence of competitors. ● = A without soil; ○ = B with soil incubated for 48 hr. before inoculation of test fungus.

with the work of Barton (1958), who has observed the growth of the fungus at different pH levels in liquid culture. Further, a feature of the experimental work of Rao (1958, 1959) which it is now possible to confirm is that, assuming that the pH value of the diffusate from the agar has not been much changed by passage through the Cellophane, then his results on the competitive saprophytic colonization of the agar plate were not appreciably biased by the use of agar acidified to pH 4·0.

The second major effect to be seen in Table 1 is the expected one, viz. a progressive decrease in fungal growth rate over the Cellophane with increase in the period of incubation of the soil inoculum on the underlying agar before the Cellophane was inoculated. The magnitude of this depression caused by the development of micro-organisms from the soil inoculum varied considerably amongst the fourteen fungi tested, and the range of variation in this respect can be seen more quickly from Fig. 1. Whereas in Table 1, only final colony diameters have been given, Fig. 1 shows, for each fungus, the increasing colony diameters plotted against time. The colony diameters shown are those on the more acid agar, (1) in the absence of soil, and (2) over agar on which soil inocula had previously been incubated for the maximum period of 48 hr.

Table 2. *Angles made with the horizontal by the straight lines representing growth/time relationships for the early growth of fourteen fungi over sterile agar* (A°) *and over agar pre-inoculated by* 48 *hr. with soil* (B°)

	A°	B°	B/A
Rhizoctonia solani	34	32	0·94
Fusarium culmorum	52	45	0·87
F. oxysporum f. *vasinfectum*	35	28	0·80
F. oxysporum f. *cubense*	40	27	0·66
F. avenaceum	47	25	0·53
Curvularia ramosa	41	21	0·51
Fusarium caeruleum	32	11	0·35
Macrophomina phaseoli	65	22	0·33
Pythium mamillatum	69	17	0·25
Ophiobolus graminis	33	8	0·24
Helminthosporium sativum	41	7	0·16
Verticillium dahliae	19	2	0·11
Helicobasidium purpureum	28	2	0·07
Fomes annosus	34	2	0·06

The growth lines A and B represent, for each fungus, straight lines which have been fitted by eye to the points representing colony diameters for the first 50 hr. of growth, line A showing the growth rate in the absence of soil (i.e. over sterile agar) and line B the rate in the presence of soil after the 48 hr. pre-incubation period. The angles made by lines A and B with the horizontal have been measured, and are given in Table 2 as A° and B° respectively.

Table 2 gives also the ratio B/A, which is a measure of the growth rate depression due to competition effects from other soil micro-organisms growing on the underlying agar, and hence is an indication of the tolerance to such competition. The fourteen fungi listed in Table 2 have been arranged in order of their tolerance factors as expressed by the ratio B/A;

the higher the tolerance, the nearer the value of B/A approaches to unity, though in no case does it attain unity.

The results of this experiment have thus provided data on the two fungal characteristics that seem most likely to influence success in competitive saprophytic colonization of the agar plate: (1) intrinsic growth rate on an agar substrate, (2) tolerance of competitive effects due to the presence of other soil micro-organisms. Further consideration of these data will be reserved for the general 'Discussion', where their significance will be assessed in relation to the results of further experiments on competitive saprophytic colonization, in extension of the work already done by Rao.

Experiment 2. This experiment was designed to determine whether the free development of bacteria from the soil inoculum, which in Exp. 1 had been restricted either by acidification of the agar to pH 4·0 or by the addition of streptomycin to the agar at pH 6·5, would appreciably increase the competitive effect of the microflora developing from the soil inoculum. The experiment was set up with a range of five test fungi, and arranged exactly as was Exp. 1, except that while both series of plates were made up with agar at pH 6·5, only one series incorporated the 0·005 % streptomycin as used before. Colony diameters given in Table 3 were measured after 35 hr. for *Macrophomina phaseoli*, and after 59 hr. for the other four fungi.

Table 3. *Colony diameters (mm.), in presence and absence of streptomycin, of five fungi on Cellophane, overlying plates of Czapek-Dox + yeast-extract agar previously inoculated with soil*

	Streptomycin present or not	No soil	Incubation period of soil (hr.)			
			0	12	24	48
Rhizoctonia solani	+	41	41	38	34	17
	−	39	37	39	29	17
Macrophomina phaseoli	+	37	36	37	32	10
	−	36	37	36	35	12
Fusarium oxysporum f. *cubense*	+	27	26	25	22	12
	−	27	26	24	20	10
Curvularia ramosa	+	26	26	23	23	16
	−	27	26	24	21	13
Ophiobolus graminis	+	16	15	17	11	4
	−	14	14	13	11	4

The results set out in Table 3 show that the greater development of bacteria permitted by omission of streptomycin has had little effect on the growth of the five test fungi. Under these conditions, therefore, competition must be chiefly interfungal.

EFFECT OF AGE OF INOCULUM ON COMPETITIVE SAPROPHYTIC COLONIZATION OF THE AGAR PLATE

Both Lucas (1955) and Rao (1959) recognized that a possible factor affecting the validity of the results of their saprophytic colonization studies with the Cambridge method or its agar plate modification was the vigour of the inoculum. They suggested that the peak of vigour might vary with the age of the culture. Rao (1959) investigated this age effect with four root-infecting fungi, but it seemed that a more critical investigation should be made into the effects of age of inoculum on the level of saprophytic colonization, using a wider range of root-infecting fungi.

Hence the purpose of the experiments to be described in this section was to confirm and amplify the results obtained by Rao with the agar plate modification of the original Cambridge method, and as the techniques he used have been fully described in a recent issue of these *Transactions*, it suffices here to give a mere outline of the modified Cambridge method as he described and used it, to facilitate an understanding of the basis of the technique. The various fungi to be tested were grown on a maizemeal + sand medium for 28 days at 25° C., after which time suitable aliquots of the pure culture were thoroughly mixed with moist unsterilized soil to give a range of inoculum dilutions. Each mixture was spread in a thin layer over the bottom of a sterile Petri dish, and over it was poured a layer of 4 % distilled-water agar cooled to 40° C. When the agar had set, 4 mm. disks were cut out of it with a sterilized no. 1 cork borer and plated out, four to a plate, on Czapek-Dox + yeast-extract agar acidified to pH 4·0 with phosphoric acid. For each series of inoculum/soil mixtures, the proportion of the total fungal growth on each plate which is subsequently occupied by the inoculant fungus decreases as the proportion of inoculum in the original mixture decreases. The *rate* of this decrease varies inversely as the competitive saprophytic ability of the inoculant fungus; i.e. vigorous saprophytes are relatively less affected by the dilution of their inocula than are weak saprophytes.

In order to standardize the above technique as far as possible, a fixed incubation period of the pure maizemeal-sand cultures had been used for the provision of inoculum, and in an experiment with *Curvularia ramosa*, *Helminthosporium sativum* (two isolates), and *Rhizoctonia solani*, Rao found that the 'peak vigour' of the inoculum from the cultures of these fungi (as assessed by its success in colonizing the agar plate against competition from other soil fungi) did vary somewhat with age, and in two of the four cultures it had declined by a significant amount after 28 days. Nevertheless, he was able to conclude that this source of variation had not been sufficient to affect the broad general conclusions derived from his main experiments employing the standard period of 28 days at 25° C. for the incubation of pure cultures. However, the source of error can be removed completely by a refinement of the original technique, whereby each fungus is tested with a range of inocula of various ages. That age of inoculum giving for a particular fungus the highest level of competitive saprophytic colonization of the test plates is selected as the age of peak vigour, and all fungi can then be compared at the peak vigour of their inocula. This variation of the original method has involved much additional labour, but it has been deemed essential in order to obtain data as reliable as possible for the correlations to be examined in the 'Discussion'.

As all eleven species of fungi to be tested could not be accommodated within a single experiment, three experiments of identical plan were carried out consecutively. Three fungi out of the fourteen studied in the present investigation were omitted from this test; *Verticillium dahliae* because it does not sporulate on the standard Czapek-Dox + yeast-extract agar used and so is difficult to identify; *Helicobasidium purpureum* because it grows so slowly, and *Fusarium oxysporum* f. *cubense* because its behaviour in Rao's experiments was very similar to that of *F. oxysporum* f. *vasinfectum* which has been included here.

Experiments 1–3. With the general object of confirming and extending the results of Rao, and the more particular one of investigating over a wider range of fungi the effects of inoculum age which he had studied for three species only, the modified Cambridge method as outlined above was adapted so that the inoculum of each test fungus was sampled for mixing with soil every 5 days over a period of 30 or more days. The incubation of the flasks as well as that of the colonized agar plates was at a temperature of 22·5° C., and they were shaken by hand every 2½ days to ensure even growth. After 7 days' incubation of the plates, recognizable areas of the inoculant fungus were recorded for the colonies on each plate, and the recording checked by a second assessment after a further 5 days. Each colony was divided into eight radial sectors, and the proportion of the total area occupied by the inoculant fungus expressed as a percentage. This method differs slightly from that of Rao, who divided each colony into four sectors, but it was not difficult to apply because a mixed fungal colony usually developed in the form of contiguous radial sectors of the different fungi composing it. For the purpose of assessing the amount of the inoculant fungus present, its eventual infiltration or overgrowth by mycelium from an adjacent sector was disregarded. The results of the experiments are set out in Tables 4–6.

Table 4. *Saprophytic colonization of agar plates against a soil microflora at different ages of inoculum*

		Age of pure culture inoculum (days)					
	Percentage inoculum	5	10	15	20	25	30
		Percentage colonization					
Fusarium culmorum	98	98	99	100	100	100	100
	90	100	100	100	100	100	100
	75	100	98	100	100	100	96
	50	100	99	100	100	100	98
	10	93	74	99	99	93	99
	Mean	98	94	100	100	99	99
Helminthosporium sativum	98	100	99	100	100	100	100
	90	98	100	100	100	100	100
	75	99	99	100	100	100	100
	50	99	88	99	100	100	100
	10	32	58	62	58	81	51
	Mean	86	89	92	99	96	90
Curvularia ramosa	98	99	100	100	100	100	100
	90	97	97	99	100	100	100
	75	91	93	96	95	86	98
	50	66	89	73	64	83	98
	10	10	22	11	8	5	10
	Mean	73	80	76	73	75	81

As indicated above, the main use of the data given in Tables 4–6 will be to provide an assessment of competitive saprophytic colonization of the agar plate by each fungal species at the strictly comparable level of peak vigour of the inoculum. But the figures can also be examined to see how far the selection of a standard period of 28 days' incubation at 25° C. is likely to have biased either the results of Rao (1959), or those of Butler (1953*a*, *b*), Lucas (1955) and Macer (1961) using the original Cambridge method with wheat straw as the test substrate for competitive saprophytic colonization.

Table 5. *Saprophytic colonization of agar plates against a soil microflora at different ages of inoculum*

		Age of pure culture inoculum (days)						
	Percentage inoculum	5	10	15	20	25	30	40
		Percentage colonization						
Pythium mamillatum	98	100	100	100	100	100	0	0
	90	100	100	100	100	100	0	0
	75	100	100	100	100	100	0	0
	50	100	100	100	100	100	0	0
	10	65	100	100	90	62	0	0
	Mean	93	100	100	98	92	0	0
Macrophomina phaseoli	98	100	100	100	100	100	100	100
	90	100	96	100	100	100	99	100
	75	100	78	95	85	99	91	95
	50	100	46	45	60	82	88	84
	10	72	3	11	12	32	62	45
	Mean	94	67	70	71	83	88	85
Rhizoctonia solani	98	79	82	65	5	59	45	9
	90	66	47	16	2	34	15	4
	75	9	19	0	0	8	8	0
	50	0	0	0	0	0	2	0
	10	0	0	0	0	0	0	0
	Mean	31	30	16	2	20	14	3
Ophiobolus graminis	98	15	10	2	0	0	0	—
	90	0	0	0	0	0	0	—
	75	0	0	0	0	0	0	—
	50	0	0	0	0	0	0	—
	10	0	0	0	0	0	0	—
	Mean	3	2	1	0	0	0	—

For seven of the eleven species tested (*Fusarium culmorum*, *F. oxysporum* f. *vasinfectum*, *F. avenaceum*, *F. caeruleum*, *Curvularia ramosa*, *Helminthosporium sativum* and *Macrophomina phaseoli*), there has been no significant decline in the colonizing vigour of the inoculum for incubation periods up to 30 days. With *Rhizoctonia solani*, the vigour of the inoculum declined somewhat with increasing age to 40 days, but there was a wide fluctuation in vigour at two of the sampling times that could not be correlated with age. It can be suggested that this might have been due to a lower growth vigour in the particular flask from which the inocula for both these mixtures were taken, but similar inconclusive results were obtained by Rao (1959) in an inoculum-age experiment with the same species. The level of colonization achieved by *Fomes annosus* was so low that no basis for comparison of the inoculum vigour at different ages is afforded by the figures. Although that reached by *Ophiobolus graminis* is but a little higher, the figures do suggest a peak of vigour in the pure culture inoculum after only 5 days' incubation. Lucas (1955) suggested that variation in inoculum vigour might be one of the causes of the discrepant values that he obtained in different colonization experiments with this fungus, but since both *O. graminis* and *Fomes annosus* produced very few recognizable colonies on the agar plate, this substrate appears to subject them to severe conditions of competition which probably mask any differences due to variability in inoculum vigour.

Table 6. *Saprophytic colonization of agar plates against a soil microflora at different ages of inoculum*

	Percentage inoculum	Age of pure culture inoculum (days)						
		5	10	15	20	25	30	40
		Percentage colonization						
Fusarium avenaceum	98	100	100	100	100	100	100	100
	90	100	100	100	100	100	100	100
	75	99	100	100	100	100	100	100
	50	95	100	100	100	100	100	100
	10	80	96	93	93	100	100	100
	Mean	95	99	99	99	100	100	100
Fusarium oxysporum f. *vasinfectum*	98	100	100	100	100	100	100	100
	90	84	100	100	100	100	100	100
	75	51	91	100	98	97	98	100
	50	28	95	91	92	87	89	88
	10	2	28	39	71	59	51	64
	Mean	53	83	86	92	89	88	90
Fusarium caeruleum	98	58	88	95	100	100	100	—
	90	16	60	65	85	76	88	—
	75	3	16	15	26	43	43	—
	50	0	4	0	11	17	18	—
	10	0	0	0	0	0	0	—
	Mean	15	34	35	44	47	50	—
Fomes annosus	98	0	10	0	16	0	0	—
	90	0	0	0	0	0	0	—
	75	0	0	0	0	0	0	—
	50	0	0	0	0	0	0	—
	10	0	0	0	0	0	0	—
	Mean	0	2	0	3	0	0	—

The remaining species, *Pythium mamillatum*, is of unusual interest, because while a mean figure of 92 % colonization for the whole range of inoculum/soil mixtures was obtained after 25 days' incubation of the inoculum, yet after 30 days' incubation (or longer), no colonization at all was obtained from any of the inocula. This surprising result was explained when inoculum from the flask 40 days old was plated out on to the acid Czapek-Dox + yeast-extract agar used in the experiment, and found to give no growth of the fungus. Microscopical examination of the inoculum showed that the original mycelium had become disorganized, and although numerous oospores were present in the aged culture these could not at this stage be induced to germinate. Subsequently mycelial growth, which must be ascribed to oospore germination, was obtained on agar from the pure culture at the age of 85 days. Nevertheless, when in an active condition, *P. mamillatum* is a vigorous competitive saprophyte, as found by Barton (1958, 1960) in his more detailed study of its saprophytic behaviour.

The results thus found for percentage saprophytic colonization are in general agreement with those of Rao for competition with Botanic Garden soil microflora, at an inoculum age of 28 days (1959, Table 14). *Helminthosporium sativum* and *Macrophomina phaseoli* have, however, behaved as much stronger competitive saprophytic colonizers, and so, to a lesser degree, have *Fusarium avenaceum* and *F. oxysporum* f. *vasinfectum*. *F. caeruleum*, on the other hand, behaved as a less vigorous colonizer of the agar plate. This was the only fungus under investigation which had perhaps not reached a peak vigour by the end of the experiment.

DISCUSSION

A consideration of the observations described above indicates that there are two possibilities for the course of the struggle for colonization of the agar plate from a mixed inoculum:

(1) The outcome is decided very quickly, before antibiotic factors come into play. In this case, one would expect to find a close correlation between the intrinsic linear growth rate of a fungus in pure culture and its success in competitive saprophytic colonization of the agar plate.

(2) The outcome of the struggle for colonization is delayed long enough to permit factors of antibiotic tolerance to be of importance. In this instance, one would expect to find some degree of correlation between success in saprophytic colonization and tolerance of antibiotic effects, and perhaps the best correlation of all between success in saprophytic colonization and fungal growth rate in the presence of competitors.

As a measure of saprophytic colonization of the agar plate, and to provide a reliable standard of comparison for each fungus, the mean saprophytic colonization ratings at peak inoculum vigour have been taken from Tables 4 to 6 for the four correlation diagrams shown in Fig. 2. These diagrams show:

Fig. 2*a*. Colonization *v.* linear growth rate in pure culture (angle A in Table 2).

Fig. 2*b*. Colonization *v.* tolerance of antibiotic effects (ratio B/A in Table 2).

Fig. 2*c*. Colonization *v.* growth rate on Cellophane film over agar *simultaneously* inoculated with soil (measured by angle, as in diagrams *a* and *d*).

Fig. 2*d*. Colonization *v.* growth rate on Cellophane film over agar pre-inoculated by 48 hr. with soil (angle B in Table 2).

In diagrams *a*, *c* and *d*, growth rate is measured as the angle made with the horizontal axis by the straight lines representing growth/time relationships for the first 50 hr. of growth.

It is evident that the best correlation is given by diagrams *a* and *c*, and that the degree of correlation between saprophytic colonization and growth rate in the presence of competitors decreases as competitive effects increase; also that the degree of tolerance to antibiotic effects gives no correlation with saprophytic colonization. It can therefore be concluded that the outcome of the struggle for colonization of the agar plate is largely decided by the relative linear growth rates of the competitors before significant antibiotic effects are produced.

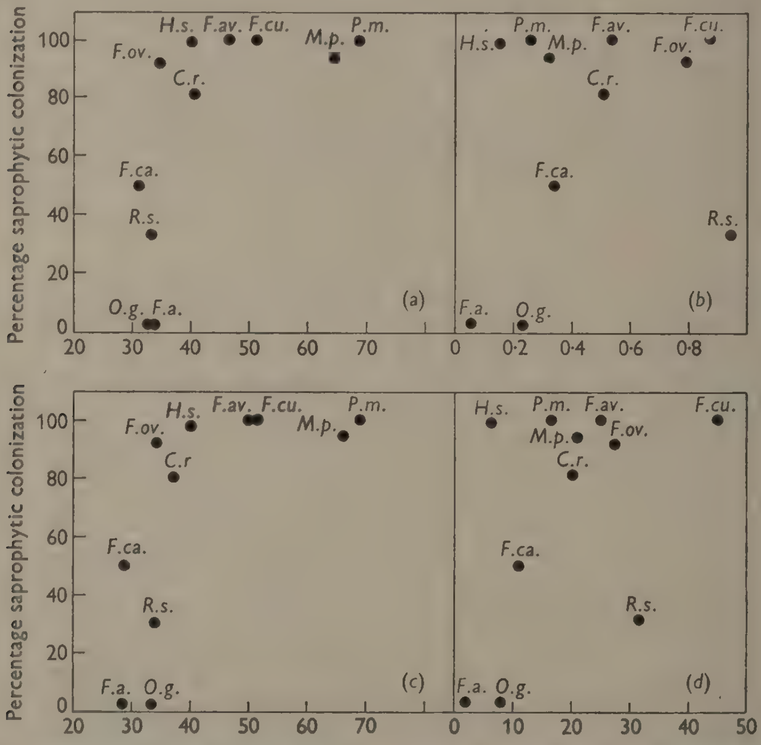

Fig. 2. Correlations between saprophytic colonization, growth rates and tolerance to antibiotic effects (for explanation see text). *C.r.*, *Curvularia ramosa*; *F.ca.*, *Fusarium caeruleum*; *R.s.*, *Rhizoctonia solani*; *F.a.*, *Fomes annosus*; *O.g.*, *Ophiobolus graminis*; *F.cu.*, *Fusarium culmorum*; *F.av.*, *F. avenaceum*; *P.m.*, *Pythium mamillatum*; *H.s.*, *Helminthosporium sativum*; *M.p.*, *Macrophomina phaseoli*; *F.ov.*, *Fusarium oxysporum* f. *vasinfectum*.

I wish to express my gratitude to Dr S. D. Garrett, who suggested the problem, for his continual guidance and advice. The work described forms part of a thesis presented for the Ph.D. degree in the University of Cambridge, and was made possible by the grant of a research studentship by the Department of Scientific and Industrial Research.

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A NEW SPECIES OF *ASPERGILLUS*

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(With 1 Text-figure)

Aspergillus brunneo-uniseriatus sp.nov., isolated from soil, is described.

During the study of fungi from soils beneath healthy and diseased shisham trees (*Dalbergia sissoo* Roxb.), a new species of *Aspergillus* was isolated on dilution plates and established in pure culture.

Aspergillus brunneo-uniseriatus sp.nov. (Fig. 1)

Coloniae in agaro Czapek floccosae, albis, latere reverso 'light green yellow'* ad 'light viridine yellow', structuris sclerotio similibus pluribus; capitula conidialia 'light mouse grey' ad 'violet grey', conidiorum catenulis radiantibus, 159–257 μ. Conidiophori hyalini, distincte septati, 252–504 × 4·5–13·4 μ, parietibus levibus, hyalinis, 0·5–1 μ crassis; vesiculae globosae, hyalinae, 9·9–19 μ; sterigmata uniseriata, hyalina, 6·1–12·8 × 4·9–6·1 μ, ad apices prolongata in tubum pallide vel alte brunneum, 6·1–28·8 × 1·8–2·4 μ, supportantem conidiorum catenulis ad apicem superiorem. Conidia globosa, primo spinulosa, evadentia irregulariter verrucosa processibus hebetibus ad maturitatem, catenulata, connectivis distinctis quae perstant conidio affixa, posteaquam conidium e catenula separatur, 'deep olive buff', parietibus 'olive brown' 6·1–12·2 μ.

Species haec nova distinguitur conidiophoris levibus, hyalinis et septatis, capitulis radiatis et brunneo-griseis, sterigmatibus uniseriatis, conidiis asperis.

Typus. Cultura in tubo, No. S20, in Culture Collection of Forest Pathology Branch, Forest Research Institute, Dehra Dun, India, deposita est.

Colonies on Czapek's agar fast growing, radial growth 2·7 cm. in 7 days at 25° C., floccose, consisting of cottony-woolly mycelium, turning woolly with age, snow-white with interspersed greyish conidial heads, reverse 'light-green yellow' to 'light viridine yellow', white sclerotia-like structures abundant. On malt agar fast growing, radial growth 2·5 cm. in 7 days, floccose with thin cottony mycelium, white with interspersed greyish conidial heads, reverse unchanged, white sclerotia-like structures abundant; conidial heads 'light mouse grey' to 'mouse grey' to 'violet grey' with radiating chains of conidia, 159–257 μ; conidiophores hyaline, distinctly septate, 252–504 × 4·5–13·4 μ, wall smooth, hyaline, 0·5–1 μ thick; vesicles globose, hyaline, 9·9–19·9 μ; sterigmata in one series, hyaline, 6·1–12·8 × 4·9–6·1 μ, prolonged at the apex into light brown to deep brown, 6·1–28·8 × 1·8–2·4 μ, tube bearing conidial chains at its upper end; conidia globose, spinulose when young, becoming irregularly warty with blunt processes with age, formed in chains with distinct connective which persists for some time on the conidium after the latter's detachment from the chain, 'deep olive buff', wall 'olive brown', 6·1–12·2 μ. White masses of vesicular hyphae suggesting the formation of sclerotia abundant in old cultures. Superficially they suggest the possibility of potential perithecia but no sign of ascospores has been found.

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* Colours referred to are taken from Ridgway (1912).

The brownish grey colour and radiate nature of conidial heads suggest the inclusion of the present species in *Aspergillus ustus* group but it differs from it in having uniseriate sterigmata and unpigmented conidiophores. Uniseriate sterigmata are found in the *A. glaucus*, *A. fumigatus* and *A. restrictus* groups. Septate conidiophores are found only in the *A. glaucus* group (Thom & Raper, 1945). But there is little in general pattern of the heads, shape and size of conidia and the colour of colonies to suggest the inclusion of this species in the above group. Hence it is described as new.

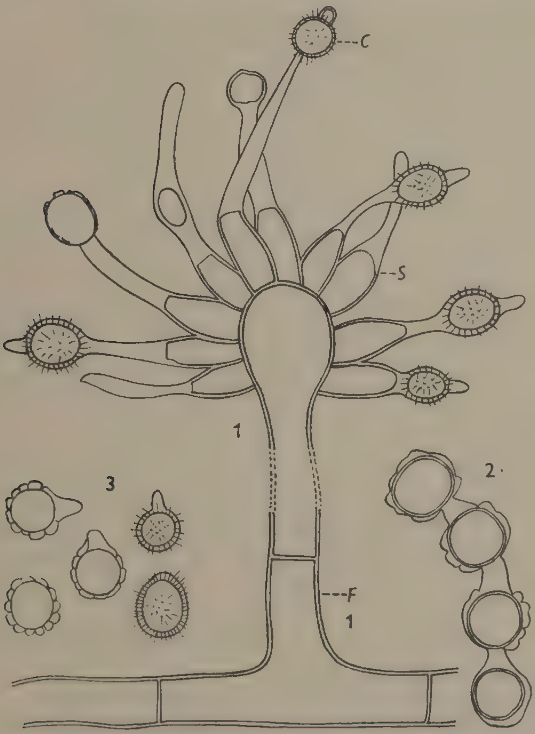

Fig. 1. *Aspergillus brunneo-uniseriatus*. 1, Conidial head with uniseriate sterigmata (*S*), conidia (*C*) and septate stalk with foot cell (*F*) (× 1500). 2, Conidial chain (× 1500). 3, Conidia (× 1500).

A culture was sent to Dr Kenneth B. Raper who replied as follows: 'This is quite unlike any *Aspergillus* that I have seen. The conidia and the radiate heads are suggestive of *A. ustus* but there is only a single series of sterigmata and the conidiophores are not pigmented. The masses of vesicular hyphae superficially suggest the possible potential of perithecia, but no sign of ascospores has been seen. There is little in the general pattern of the heads to suggest the *A. glaucus* or *A. fumigatus* groups with uniseriate sterigmata. This, I believe, represents a new species.'

Our sincere thanks are due to Dr K. B. Raper for his invaluable advice and to Rev. Dr H. Santapau for rendering the Latin diagnosis of the new species.

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STUDIES ON BRITISH CHYTRIDS

XVII. SPECIES OCCURRING ON PLANKTONIC DESMIDS

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(With Plate 12 and 4 Text-figures)

Rhizophydium ubiquetum sp.nov., *R. chaetiferum* Sparrow, *R. gibbosum* (Zopf) Fischer and *Phlyctochytrium planicorne* Atkinson are described from Windermere, living on planktonic desmids.

In Windermere, in the English Lake District, there are about fifteen species of common planktonic desmids. None of them escapes parasitism by one or, in some cases, several species of chytridiaceous or holocarpic biflagellate fungi.* So far, owing to the failure to observe important phases in the life history, only two of the chytridiaceous parasites have been described, *Rhizophydium difficile* Canter (1954) and *Chytridium isthmiophilum* Canter (1960). However, many of the desmids provide a substratum for saprophytic chytrids, following on after a parasitic infection. It is with these saprophytic species that this paper is concerned. The records of the dates when the chytrids were observed in plankton collections (Tables 1–4) are based on samples collected at approximately weekly intervals from 1947 to 1959, with the exceptions of 23 July–13 August 1951, 27 July–7 September 1953 and 20 November 1958–5 February 1959. Where appropriate, records from other lakes in the English Lake District are included.

Rhizophydium ubiquetum sp.nov. (Text-figs. 1 *a–s*, 2 *a–k*, and Pl. 12)

Thallus monocentricus, eucarpicus, e sporangio sessili vel non sessili epibiotico, e zoospora encystata evoluto. Sporangia non-sessile ex axe rhizoideo epibiotico, et rami laterales interdum enascuntur constantia. Alia rhizoidea e membrana sporangii interdum enascuntur. Systema endobioticum rhizoideum ramosum. Sporangium plus minusve piriforme, 4–14 μ lat., 5–18 μ alt., papilla singula lata apicali (raro basali aut laterali) membranam tenuem habente, 1–30 zoosporas continentem. Zoosporae 4·5 μ diam., globulum olei singulum basalem atque flagellum 25 μ long. habentes, in massa immobili enascentes, quasi confestim mobili facta. Spora quiescens asexualis, sphaerica, 9–15 μ diam., membranam crassam levem sine colore habens, globulos refractivos unum magnum atque multos minores continentem. Systema rhizoideum illi sporangiali simile, nullis ramis autem, e membrana sporae enascentibus. Germinatio ignota.

Planta saprophytica in *Cosmario contracto* Kirchn. var. *ellipsoideo* (Elfv.) West et West (substrato typi) atque in aliis desmidiis unicellularibus in plancto lacus Windermere dicti (loci scilicet typi) atque aliorum lacuum in regione lacustri Anglica. Typus Herb. I.M.I. No. 83244.

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* There are three biflagellate fungi which commonly parasitize desmids in Windermere, but so far only two have been identified: *Myzocytium megastomum* De Wildeman (Canter, 1947) and *M. proliferum* Schenk.

Thallus monocentric, eucarpic, epibiotic sporangium sessile or non-sessile developed from the encysted zoospore. Non-sessile sporangia with an epibiotic rhizoidal axis from which lateral branches may arise. Other rhizoids may grow from the sporangium wall. Endobiotic rhizoidal system branched. *Sporangium* more or less pyriform, 4–14 μ broad and 5–18 μ high, with a single broad thin-walled apical (rarely basal or lateral) papilla, containing 1–30 zoospores. *Zoospores* 4·5 μ diam. with a single basal oil globule and flagellum 25 μ long, emerging in a non-motile mass and almost immediately becoming motile. Resting spore asexual, spherical, 9–15 μ diam., with a thick smooth colourless wall; content one large and many smaller refractive globules. Rhizoidal system similar to that of sporangium but no branches arising from spore wall. Germination unknown.

Table 1. *Records of* Rhizophydium ubiquetum *sp.nov.* from *all substrata*

Windermere: North basin

1947	21 July, 29 Sept., 6, 20, 27 Oct., 3 Nov.
1948	7 June, 19, 26 July, 13–27 Sept., 11–25 Oct., 1, 8, 29 Nov., 6, 20 Dec.
1949	9 Jan., 18, 30 July, 3, 9 Aug., 13, 19, 26 Sept., 3 Oct.–15 Nov.
1950	3 Jan., 17, 31 July, 14–28 Aug., 18, 26 Sept., 3 Oct.–27 Nov., 11 Dec.
1951	27 Aug., 3–24 Sept., 8 Oct.–31 Dec.
1952	4 Feb., 3 Mar., 7 Apr., 21 July, 5 Aug.–27 Oct., 10, 17 Nov.
1953	27 July, 28 Sept., 23, 30 Nov., 21 Dec.
1954	18 Jan., 2, 22 Feb., 15 Mar., 3, 16 Aug., 6 Sept.–18 Oct.
1955	24 Oct.–7 Nov., 12 Dec.
1956	30 Jan., 20 Aug., 3, 10, 24 Sept., 1, 8, 22 Oct., 19 Nov.
1957	23, 30 Sept., 7 Oct.–11 Nov.
1958	18 Aug., 2, 9, 23 Sept., 7 Oct.–18 Nov.
1959	29 Sept., 6, 27 Oct., 1, 10 Nov.

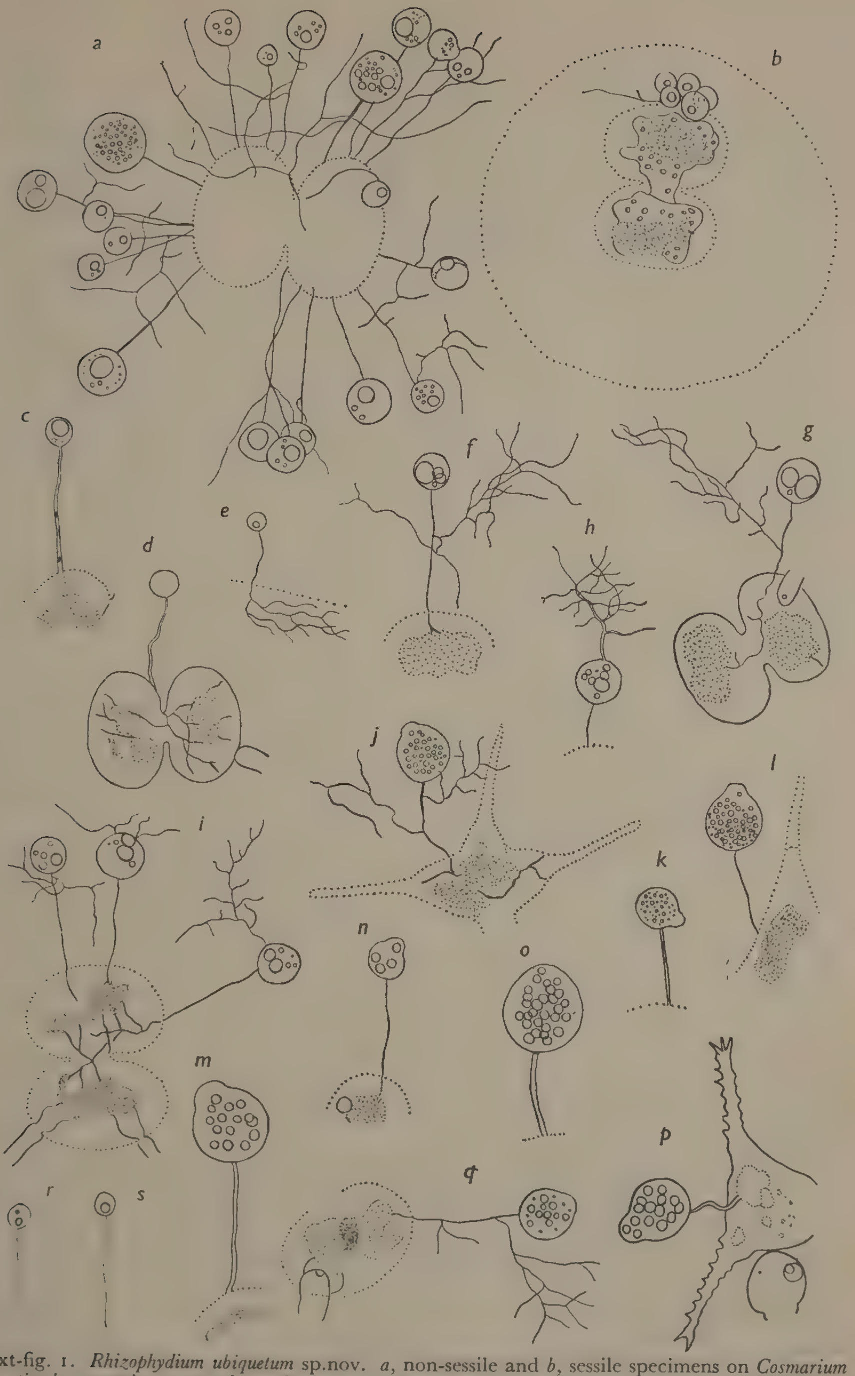
Windermere: South basin

1947	7, 14 Oct.
1948	8, 22, 30 June, 5 July, 14, 22 Sept., 1, 5, 19, 26 Oct., 23 Nov., 14 Dec.
1949	9, 16 Aug., 5 Sept.–25 Oct.
1950	10–30 Oct., 21, 27 Nov., 4, 27 Dec.
1951	2 Jan., 13 Aug., 3–24 Sept., 8–30 Oct., 5, 19, 26 Nov., 3, 10 Dec.
1952	16–30 June, 7, 14 July, 5 Aug.–6 Oct., 27 Oct.–15 Dec.
1953	14 Sept., 26 Oct., 3 Nov.–30 Dec.
1954	3, 23, 30 Aug., 3 Sept.–1 Nov., 6 Dec.
1955	14 Nov.
1956	16 Jan., 25 June, 9, 17 July, 20 Aug., 29 Oct.
1957	21, 28 Oct., 4–18 Nov.
1958	29 July, 14–28 Oct., 4, 11 Nov.
1959	20 Oct.–17 Nov.

Saprophytic on *Cosmarium contractum* Kirchn. var. *ellipsoideum* (Elfv.) West & West (type substratum) and other unicellular desmids, in the plankton of Windermere (type locality) and other lakes in the English Lake District. Type specimen (slides) in Herb. I.M.I. no. 83244.

This chytrid is widespread in the English Lake District as a saprophyte on many species of planktonic desmids. It is recorded from Buttermere, Crummock Water, Derwent Water, Ennerdale, Esthwaite Water, Hawes Water, Loughrigg Tarn, Loweswater, Ullswater and Windermere. The following account is based entirely on the Windermere material.

In this lake *Rhizophydium ubiquetum* grows on *Cosmarium contractum* var.



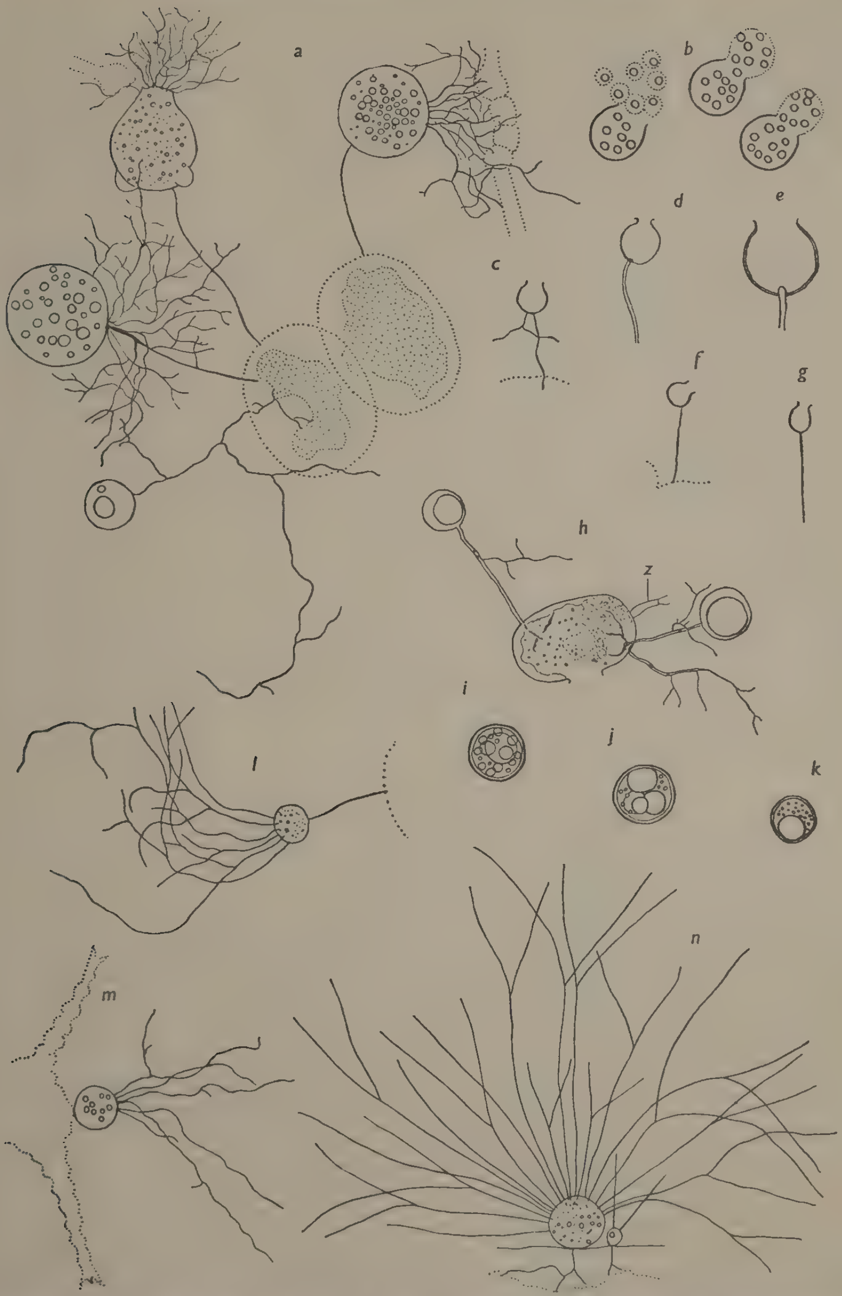
Text-fig. 1. *Rhizophydium ubiquetum* sp.nov. *a*, non-sessile and *b*, sessile specimens on *Cosmarium* (in *b*, note the unequal mucilage development of the desmid and the internal thallus of *Myzocytium*); *c*, expanded zoospore, epibiotic portion of germ thread with refractive material; *d*, young thallus with branched endobiotic rhizoids; *e*, ? specimen of this fungus on *Spirogyra*; *f–i*, young sporangia with varied types of epibiotic rhizoidal development; *j–l*, immature sporangia each with a dehiscence papilla; *m*, papilla region with dense hyaline material; *n–p*, mature sporangia; *q*, mature sporangium, zoospores with globule and granule; *r*, zoospore from latter sporangium; *s*, more usual zoospore. In *d*, *g*, *i* and *q* can be seen the exit tubes of *Myzocytium proliferum*. All ×650.

ellipsoideum, *Staurastrum cingulum* (West & West) Smith, *S. lunatum* Ralfs, *S. anatinum* Cooke & Wills, *S. megacanthum* Lundell, *S. planktonicum* Teiling, *S. pseudopelagicum* West & West, *Spondylosium planum* (Wolle) West & West, *Staurodesmus curvatus* (W. West) Teiling and *Xanthidium subhastiferum* W. West. It is usually found as a secondary invader following a previous infection by chytridiaceous or holocarpic biflagellate fungi. Occasionally it is found on desmids which have died from some cause other than parasitism. It is of interest that frequently the thallus of the biflagellate contained in a desmid which becomes infected by *R. ubiquetum* fails to mature and dehisce.

Most planktonic desmids are surrounded by a mucilage envelope which varies in thickness according to the species. Where it is usually relatively wide (e.g. *C. contractum*) the infecting zoospore may encyst at any point from the edge of the mucilage to directly on the algal wall. The latter position is much less frequently encountered here than in desmid species where the mucilage envelope is comparatively narrow (e.g. *Staurastrum cingulum*). On germination the zoospores not directly attached to the desmid wall produce an epibiotic germ thread (Pl. 12, fig. 1) the length of which varies according to the distance of the zoospore from the cell wall. On further development the germ thread may widen slightly (Text-fig. 1 *c*) and sometimes rhizoidal branches arise from it at one or more places (Text-fig. 1 *f*, *g*). These rhizoids permeate the mucilage and may trail into the water beyond. Frequently no such branches develop and the germ thread remains as a stalk-like portion of the thallus (Text-fig. 1 *m–o*). In a few specimens rhizoidal-like branches have been found growing from the wall of the sporangium (Text-fig. 1 *h*, *i*). The endobiotic rhizoidal system is often difficult and sometimes impossible to see. This may be because of the dense algal chloroplast or the presence of an endobiotic biflagellate thallus (Pl. 12, fig. 5). When visible it appears as a well branched system ramifying through both semi-cells (Text-fig. 1 *d*, *i*). In both sessile and stalked types of thalli the endobiotic rhizoids may re-emerge from the desmid cell out into the mucilage envelope.

The zoospore enlarges into the sporangium which is spherical at first but later becomes more pear-shaped by the development of a broad apical papilla (Text-fig. 1 *m*). Occasionally a lateral papilla is formed (Text-fig. 1 *k*) and one sporangium was seen which bore an apical tuft of rhizoids and had developed two basal dehiscence papillae (Text-fig. 2 *a*). The original globule of the zoospore enlarges and remains conspicuous during the early growth of the sporangium, but later it becomes lost in the mass of other accumulating globules. When mature, the refractive globules of the individual zoospores tend to be arranged centripetally in a greyish granular matrix; the papilla region now contains dense hyaline protoplasm. The majority of mature sporangia range from 10–18 μ high × 8–14 μ broad and contain from 4 to 30 zoospores. Minute empty sporangia 5 × 4·6 μ (Text-fig. 2 *f*, *g*), in which there could have been only one or two zoospores, have also been found.

Before dehiscence the content of the papilla becomes less dense. On dehiscence the tip dissolves and the sporangial content flows out in a mass (Text-fig. 2 *b*). Immediately, the individual zoospores separate and swim



Text-fig. 2. *a–k*, *Rhizophydium ubiquetum* sp.nov. *a*, one sporangium has two basal dehiscence papillae; note rhizoids adherent to cells of *Asterionella*; *b*, dehiscence; *c–g*, empty sporangia; *h*, (?) two young resting spores; *i*, *j*, immature and *k*, mature resting spore. *l–n*, *R. chaetiferum* Sparrow. *l*, non-sessile; *m*, *n*, sessile sporangia (*n*) on *Spirogyra*. In *h*, the desmid contains a *Myzocytium* thallus; on the outside at (*z*) are the remains of its germ tube and appressorium. *a*, ×800; rest, ×650.

off with a smooth gliding motion. The zoospore is spherical with a conspicuous basal globule, sometimes a weakly refractive granule can also be distinguished (Text-fig. 1*r*). The empty sporangium is persistent and on either side at its apex can be seen thinner walled, outwardly curving portions (Text-fig. 2*c*–*g*). These probably represent the remains of the papilla wall. Often at the base of the empty sporangium can be seen a refractive septum (Pl. 12, fig. 6) which in larger sporangia is dome shaped (Text-fig. 2*e*).

The spherical resting spore is formed asexually by direct enlargement of the encysted zoospore. When young it is virtually indistinguishable from a sporangium, except perhaps for the greater accumulation of refractive material in the form of many unequal-sized globules or of a single very large one (Text-fig. 2*h*). At maturity it is surrounded by a thick smooth colourless wall and the content is dominated by a single large globule (Pl. 12, fig. 7; Text-fig. 2*k*). To date no resting spore sessile on a desmid cell has been found. The portion of the germ thread connecting the resting spore to its endobiotic rhizoidal system usually remains stalk-like and lateral branches rarely arise from it. No rhizoids have been seen growing from the wall of the resting spore itself. Its method of germination is unknown.

This chytrid exhibits a great diversity in its epi-interbiotic relationships to the substratum and in the nature of its rhizoidal system. The sporangium may be epibiotic and sessile or, when part of the germ thread is epibiotic, stalked. Again, thalli with branched rhizoids external to the substratum occur. These rhizoids arise either laterally from the original germ thread or may be rhizoidal-like prolongations from the wall of the sporangium. Sometimes these rhizoids from the sporangium and those from the germ tube have been found adherent to other debris in the sample (e.g. dead *Asterionella* cells). Similar variations of the thallus to the substratum were noted for *Phlyctochytrium punctatum* by Koch (1957) who stated that they also exist to a lesser degree in *P. irregulare*.

Branched rhizoid-like prolongations from the wall of the sporangium were found in certain isolates of *P. punctatum* (Koch, 1957, p. 114). Further, Ajello (1945) described the occasional proliferation of the apices of the teeth in *P. aureliae*. *Rhizophlyctis harderi* Uebelmesser (1956) also develops epibiotic prolongations of the thallus. Uebelmesser described two types of thalli, one thick-walled (? the resting spore) bearing long apical strands which could attach themselves to other pollen grains. From these so-called resting spores in culture arise thin-walled sporangia with or without branched epibiotic prolongations from the wall.

Many specimens of *Rhizophydium ubiquetum* could be placed in *Rhizidium* as it is at present defined. It is clear that the importance given to the broad main epibiotic rhizoidal axis as a distinguishing feature in this genus is doubtful. The swarming of the zoospores in a vesicle before dehiscence may prove a more trustworthy generic character in *Rhizidium*. Since this is not a characteristic of the present fungus I am assigning it to *Rhizophidium*.

Serbinow (1907)* assigns to *Rhizidium braunii* Zopf (1888) a chytrid saprophytic on bog desmids after infection by *Rhizophydium globosum. Rhizidium braunii* infects diatoms in salt pools and because of these differences in substratum and habitat, as well as the fact that little is known about Serbinow's fungus, his identification is considered doubtful. In many respects his fungus resembles *Rhizophydium ubiquetum.* It is saprophytic on desmids, the zoospore encysts in the mucilage and produces a germ thread to the algal wall. Serbinow's figure (Text-fig. 3*e*) shows that rhizoidal branches arise from both the germ thread and the sporangium wall. The shape of the mature sporangium is unknown and resting spores have not been observed. However, on the available evidence it seems that Serbinow's fungus is closer to *R. ubiquetum* than to *Rhizidium braunii.*

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* There are discrepancies between the Russian and German descriptions to the figures. The Russian is clearly the correct one.

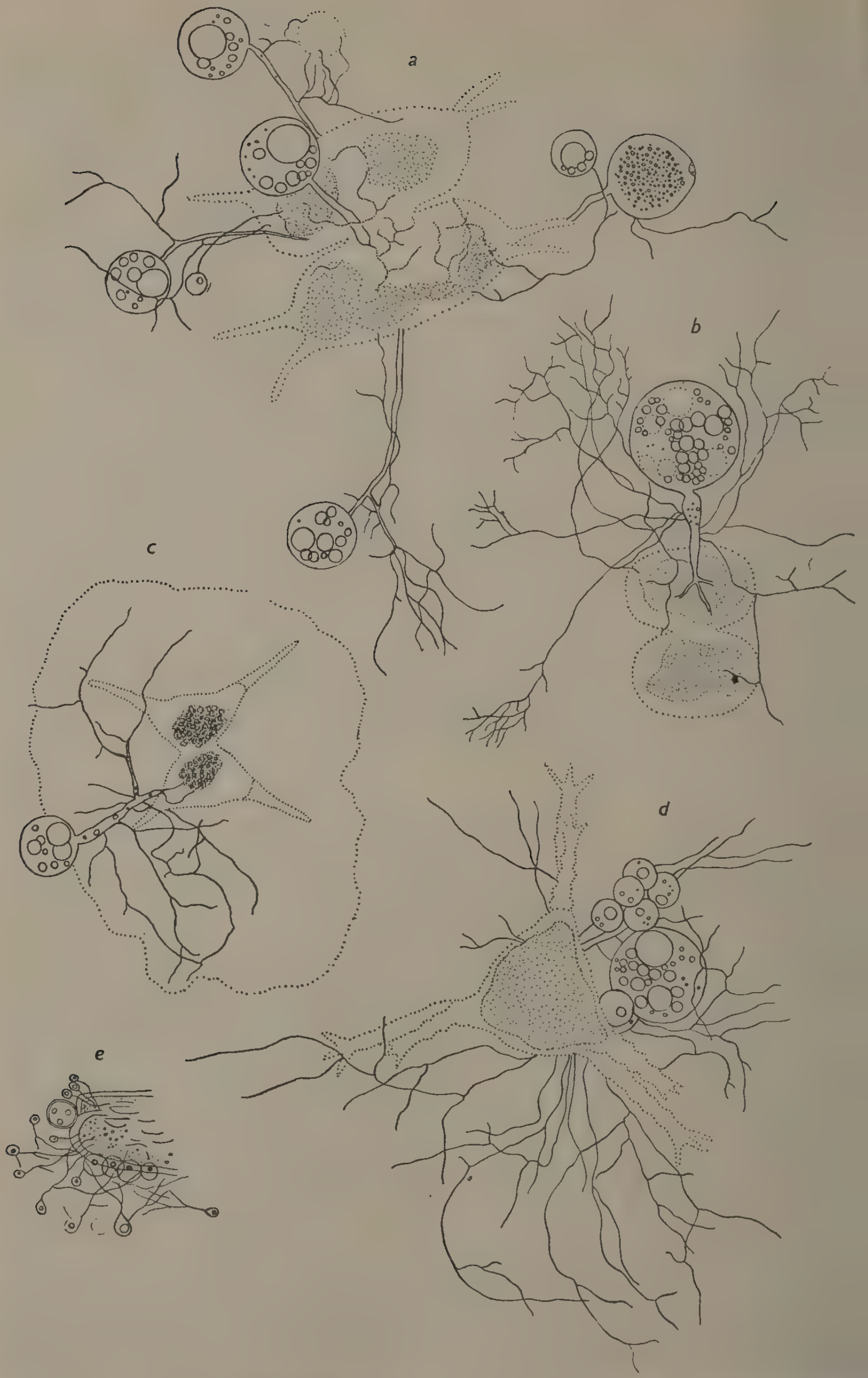
When species of *Rhizophydium* were examined it became obvious how closely *R. ubiquetum* resembles *R. pollinis-pini* (Braun) Zopf *pro parte.* It is conceivable that in the future *R. ubiquetum* may prove to be a synonym of *R. pollinis-pini*, the variations in the thallus structure of the former resulting from the different substrata invaded. Thalli resembling *R. ubiquetum* have been found on dying fragments of *Spirogyra* from Windermere collected at the same time as the desmids in the plankton sample. Again, what is probably this chytrid has been seen in the mucilages of the colonial green algae *Gemellicystis neglecta* (Teiling) Skuja, *Sphaerocystis schroeteri* Chodat and *Eudorina elegans* Ehrenb., after they have been attacked by parasitic fungi in Windermere. These observations suggest that *R. ubiquetum* may be a general saprophyte of algae. If this species is obtained in culture then its ability to grow on pollen can be tested, which in turn may clarify its possible relationship with *R. pollinis-pini.* At present it must be considered as a separate species.

Thalli of uncertain relationships (Text-fig. 3*b–d*)

Very rarely in samples from Windermere containing desmids with *R. ubiquetum* are to be found thalli whose relationships to this chytrid remain uncertain. Details of the mature sporangium, dehiscence and resting spore formation are lacking. When young these thalli are indistinguishable from *R. ubiquetum.* However, as development proceeds the epibiotic germ thread of non-sessile specimens broadens and becomes more like a main rhizoidal axis (Text-fig. 3*b*, *c*), while the production of epibiotic rhizoids in the mucilage would appear more constantly greater than in *R. ubiquetum.* The sporangia also are larger, up to 27 × 25 μ. It may be that these thalli belong to a different species but equally they could be interpreted as giant specimens of *R. ubiquetum.* Sparrow (1938) noted an abnormal sporangium in *Phlyctochytrium urceolare* Sparrow which was at least twice the size of other ones.

RHIZOPHYDIUM CHAETIFERUM Sparrow (Text-fig. 2*l–n*)

This fungus is occasionally present in small numbers in Windermere and was found in Loweswater in 1949. The few specimens observed agree well with the description given by Sparrow (1939) though neither dehiscence nor resting spores have been seen. Sporangia range from 9 to 13 μ diam., and the branched hairs may extend up to 75 μ in length. Like *R. ubiquetum*



Text-fig. 3. *a*, ? thalli of *Rhizophydium ubiquetum*; some may be young resting spores; *b–d*, thalli of uncertain relationships; *e*, *Rhizidium braunii* Zopf; the spherical sporangia on the desmid wall belong to *Rhizophydium globosum* (after Serbinow, 1907). *a*, ×800; *b–d*, ×650.

thalli most frequently occur in Windermere on the planktonic desmids which have been previously infected by fungi. *R. chaetiferum* has been found on *Cosmarium contractum* var. *ellipsoideum*, *Staurastrum cingulum*, *S. planktonicum*, *S. lunatum*, *S. anatinum*, *S. pseudopelagicum* and *Xanthidium subhastiferum*. Again, it was once found on *Asterococcus* sp., and fragments of dead *Spirogyra*. In Loweswater it occurred on *Staurastrum cingulum*, *S. lunatum* and *Spondylosium planum*.

Table 2. *Records of* Rhizophydium chaetiferum *from all substrata*

	Windermere	
Year	North basin	South basin
1947	—	18 June
1950	17–31 July	4–10 Sept.
1951	18 June	20, 27 Aug., 10, 24 Sept.
1952	14 July, 11 Aug.	16 June, 21 July–28 Aug.
1959	6, 27 Oct.	22 June, 6 Oct.

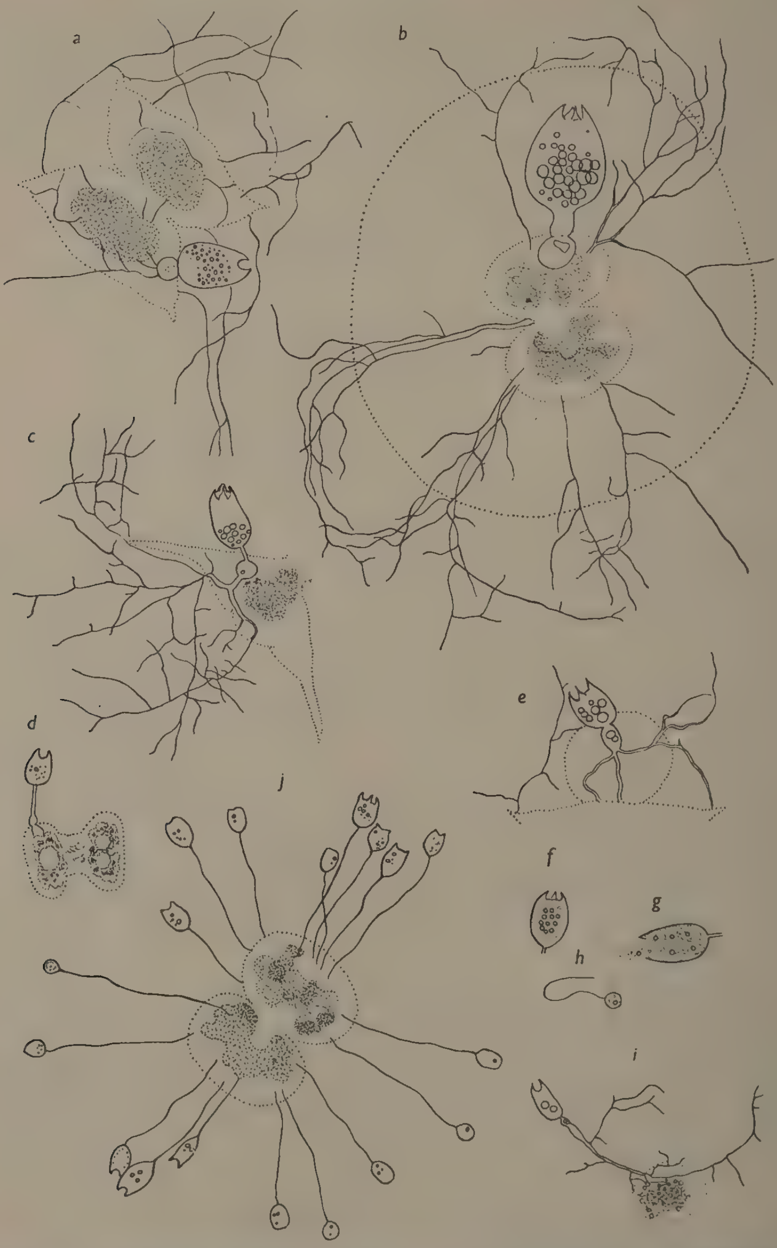
All the records of *R. chaetiferum* by other workers, Fott (1951), Karling (1941) and Sparrow (1939, 1952), concern specimens growing on algae which normally inhabit the littoral regions of lakes or live in bogs, e.g. *Cladophora*, *Oedogonium*, *Bulbochaete*, *Spirogyra* and *Chara*. It seems likely therefore that this chytrid is not truly planktonic. Probably infected fragments of littoral algae or indeed pollen grains (see Scholz later) become washed out into the lake and if conditions are suitable infection is transferred to dying planktonic algae. This could account for its sparsity in the plankton.

Again, in all previous descriptions the sporangia are said to be sessile. In the Windermere material, while the majority of sporangia were sessile on the wall of the infected alga, stalked specimens do occur. These are formed when a zoospore encysts in the mucilage of a desmid some distance from its wall. The epibiotic portion of the germ-tube which forms this stalk remains more or less unaltered (Text-fig. 2*l*) and never bears lateral branches.

The records so far mentioned are all from freshwater habitats. However, Scholz (1958) records it on bait of *Pinus* pollen from soil collected at various places on bare sand on the Mediterranean coast (Mallorca). Scholz found that although the sea shore sand was not very salty (water soluble salt represented 0·14% dry weight), in the laboratory the chytrid did grow in solutions of NaCl and $MgCl_2$ of up to 0·5 mol./l.

PHLYCTOCHYTRIUM PLANICORNE Atkinson (Text-fig. 4*a–j*)

Once again, this is a species found on dying planktonic desmids, namely *Cosmarium contractum* var. *ellipsoideum*, *Staurastrum anatinum*, *S. lunatum*, *S. megacanthum*, *S. planktonicum*, *Staurodesmus curvatus* and *Spondylosium planum*. Occasionally specimens also occurred on *Asterococcus* sp., *Sphaerocystis schroeteri* and *Peridinium cinctum* (O.F.M.) Ehrenb. One thallus



Text-fig. 4. *Phlyctochytrium planicorne* Atkinson. *a–d*, immature sporangia on various desmid
e, thallus with apophysis and rhizoids embedded in a chytrid sporangium growing on th
desmid; *f*, mature sporangium; *g*, escaping zoospores; *h*, zoospore; *i*, young thallus growin
in the mucilage of and attached to a cell of *Gemellicystis*; *j*, a *Cosmarium* infected by man
abnormally developed, non-sessile thalli. All ×650.

shown (Text-fig. 4*e*) penetrated through and killed a sporangium of *Rhizophydium* sp., which was parasitizing *Staurastrum lunatum*.

While the sporangium and its apical collarette of four plain teeth seems to be a fairly constant feature of this chytrid, considerable morphological variation is shown in the swelling beneath the sporangium and in the rhizoidal system. Both stalked and sessile sporangia are produced and the stalk may remain isodiametric or become somewhat swollen. In the Windermere material thalli mostly had an apophysis, which was either immediately below the sporangium (Text-fig. 4*a*, *b*) or separated from it by an intervening stalk-like portion (Text-fig. 4*c*, *d*). From the apophysis the main rhizoidal axis (or axes) diverges. Sometimes the rhizoids ramify inside a desmid and then appear to emerge again into the surrounding mucilage (Text-fig. 4*b*). Early in the expansion of the zoospore cyst into the sporangium the four apical teeth become visible. During growth the protoplasm contains a few globules, often grouped in a cluster. At maturity the globules of the incipient zoospores are grouped in a central position with greyish cytoplasm surrounding them (Text-fig. 4*f*). The sporangia (excluding the teeth) range from 11–26 μ high × 8–18 μ broad.

Table 3. *Records of* Phlyctochytrium planicorne *from all substrata*

	Windermere	
Year	North basin	South basin
1949	30 July	—
1950	—	28 Aug.
1951	—	3 Sept., 30 Oct., 26 Nov., 3, 31 Dec.
1952	25 Aug., 22, 29 Sept.	21, 28 July, 5 Aug.–2 Oct.
1958	28 Oct.	—

On dehiscence a portion of the apex of the sporangium dissolves and part of the protoplasm, together with some of the oil globules, emerges in an undifferentiated mass. The neck of the sporangium appears stretched to capacity (see also Sparrow, 1932, fig. 3*e*), the mass of protoplasm outside being continuous with that left inside the sporangium. Almost at once outlines of the zoospores are seen, flagella soon appear and within 5 min. the zoospores are actively swimming. It is possible that the zoospore mass is surrounded by a delicate vesicle though I did not observe it. The sporangium often collapses before all the zoospores have emerged and those thereby trapped come out slowly in an amoeboid manner, becoming elongated as they pass through the apex. They then shoot through the mucilage zone of the alga into the water where they round off and swim away. Their movement is smooth and gliding, with frequent changes in direction. The zoospore is 4 μ diam. with a single refractive globule and a flagellum about 30 μ long. The thalli pictured in Text-fig. 4*j* are presumably those of *P. planicorne* though the apophysis and branched rhizoidal system, if any, attached to them could not be found.

P. planicorne has been recorded in the United States of America on a wide variety of filamentous green algae, such as *Spirogyra*, *Cladophora*, *Rhizoclonium* and *Oedogonium* (Atkinson, 1909; Sparrow, 1932, 1938, 1952).

It is also recorded by Sparrow (1943, p. 230) on decaying stems of *Acorus calamus*. Once again it seems probable that the source of infection for the planktonic algae originates in the littoral regions.

Rhizophydium gibbosum (Zopf) Fischer

The records of this chytrid were on *Staurastrum anatinum*, *S. pseudopelagicum*, *Staurodesmus curvatus* and *Staurodesmus* sp. As only empty sporangia were seen it is impossible to say whether it is parasitic or saprophytic. In a collection from Ullswater (West), 6 February 1952, it was found on *S. jaculiferus* (W. West) Teiling. Again, I have found it growing on a variety of non-planktonic desmids in the bog bordering the northern edge of Blelham Tarn, Blelham Tarn fish pond and in Rusland Moss near Newby Bridge, all in north Lancs. *R. gibbosum* has been described from a wide variety of substrata, including algae belonging to the Conjugales, protozoan cysts, and rotifer eggs. The sporangia I have seen most closely resemble those figured by Zopf (1888) on *Penium* and by Rieth (1954) on *Spirogyra*. In both the Rusland and Blelham fish pond material empty sporangia were found with secondary sporangia growing from the base. It may be that this chytrid exhibits internal proliferation. It has previously been recorded from England (Sparrow, 1957) living on protozoan cysts.

Table 4. *Records of* Rhizophidium gibbosum *on planktonic desmids*

	Windermere	
Year	North basin	South basin
1949	3, 17 Jan.	—
1950	6 Feb.	—
1956	30 Jan.	16, 30 Jan.
1959	—	10 Feb.

There are few records of chytrids on planktonic desmids in lakes and other large bodies of water. Apart from *Rhizophydium difficile* and *Chytridium isthmiophilum*, the only other record for Great Britain is of an undetermined species on *Staurastrum paradoxum* Meyen in Swithland Reservoir, Leics. (Reynolds, 1940). Paterson (1958) described two incompletely known chytrids from lakes in Michigan, U.S.A.: the first, referred to as 'Species 3' was parasitic on *Staurastrum* sp. in Frains Lake and the second, 'Species 4', was parasitic on *Closterium* sp. in Crooked Lake. From South America Karling (1944) recorded *R. globosum* (Braun) Rabenhorst growing on *Xanthidium subhastiferum* in the swimming pool of the Bosque Club at Manaus. The sporangia in Karling's material were infected by *Rozella rhizophydii* Karling. *Olpidium endogenum* (Braun) Schroeter is described by Skuja (1948, 1956) from lakes in Sweden. It occurred in *Cosmarium depressum* (Naëg) Lund and its var. *achondrum* (Boldt) W. & G. S. West. As it is not stated in the text whether zoospores were seen, Skuja's figure could equally be interpreted as a dwarf form of *Myzocytium megastomum*.

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EXPLANATION OF PLATE 12

Rhizophidium ubiquetum sp.nov. on *Cosmarium contractum* Kirchn. var. *ellipsoideum* (Elfv.) West & West from Windermere

Fig. 1. Germinating zoospores and young thalli.
Fig. 2. At (*M*) empty zoospore case, germ-tube and appressorium of the parasite *Myzocytium*.
Fig. 3. Germinated zoospores and immature sporangia.
Fig. 4. A mature sporangium (*Sp*), slightly out of focus.
Fig. 5. Young thalli: one has produced lateral rhizoids through the mucilage envelope of the desmid and made contact with an *Asterionella* cell, itself bearing a chytrid sporangium. Inside the *Cosmarium* is a large undehisced specimen of *Myzocytium*.
Fig. 6. An empty sporangium; refractive septum at (*S*).
Fig. 7. A resting spore; situated behind it is an empty sporangium.

The specimens were all mounted in indian ink to show more clearly the mucilage envelope of the desmid. Fig. 5, × 480; rest, × 730.

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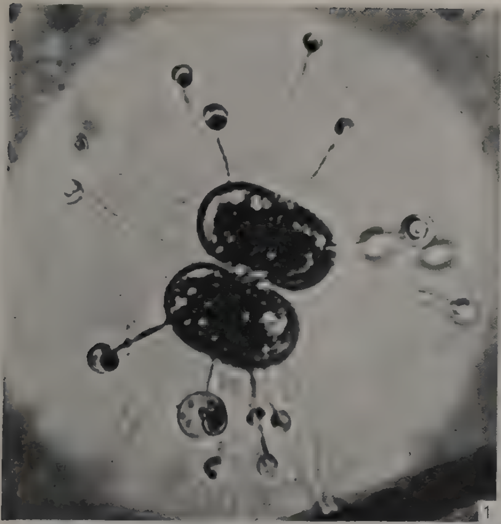
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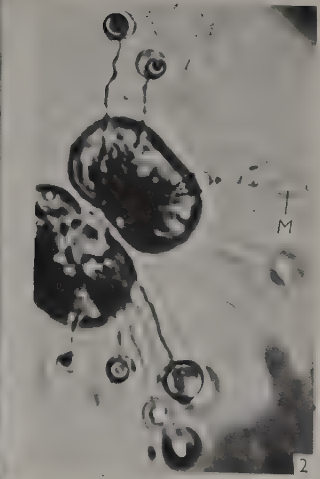

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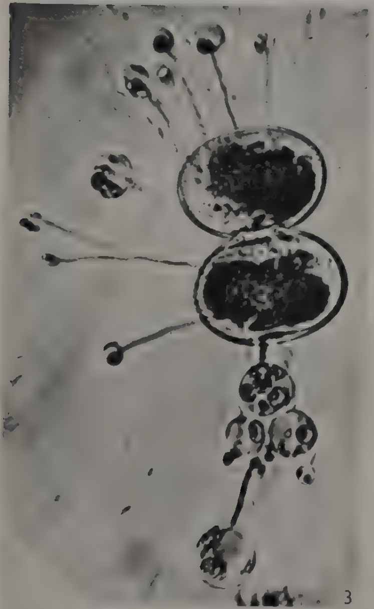
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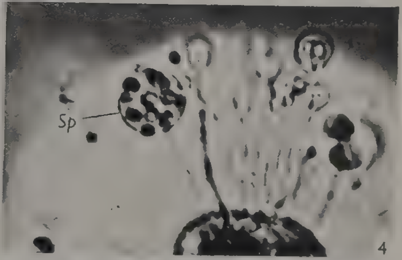

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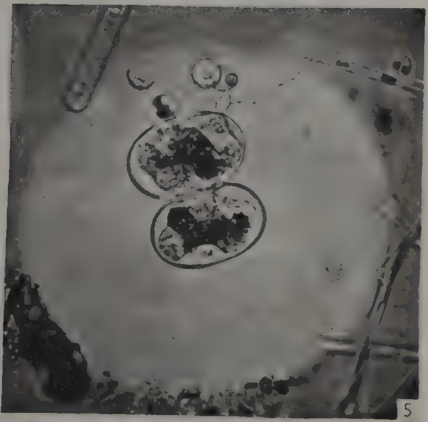
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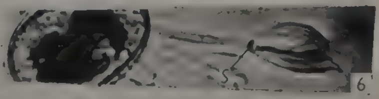

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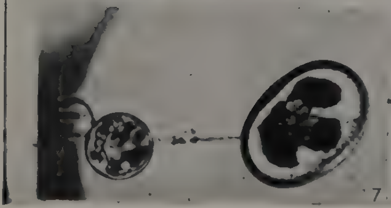
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Trans. Brit. mycol. Soc. **44** (2), 177–184 (1961).

TWO NEW SAPROPHYTIC CHYTRIDS FROM THE LAKE DISTRICT

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(With Plates 13 and 14 and 3 Text-figures)

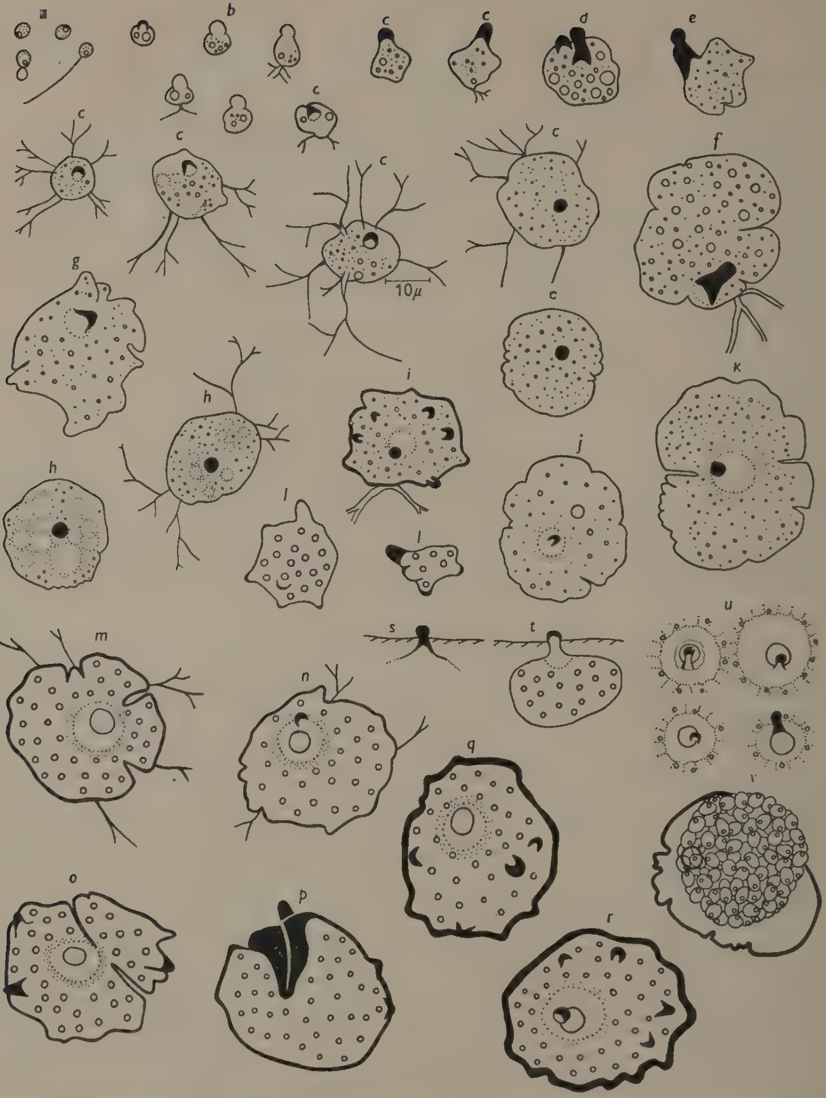
Entophlyctis lobata sp.nov. and *Chytriomyces poculatus* sp.nov. are described and illustrated.

The two species here described were obtained in the course of work on the distribution of lower saprophytic fungi (particularly Chytridiales) in the English Lake District. Both aquatic and terrestrial environments are being sampled in this survey, the fungi being recovered by baiting mud or soil with suitable organic substrata.

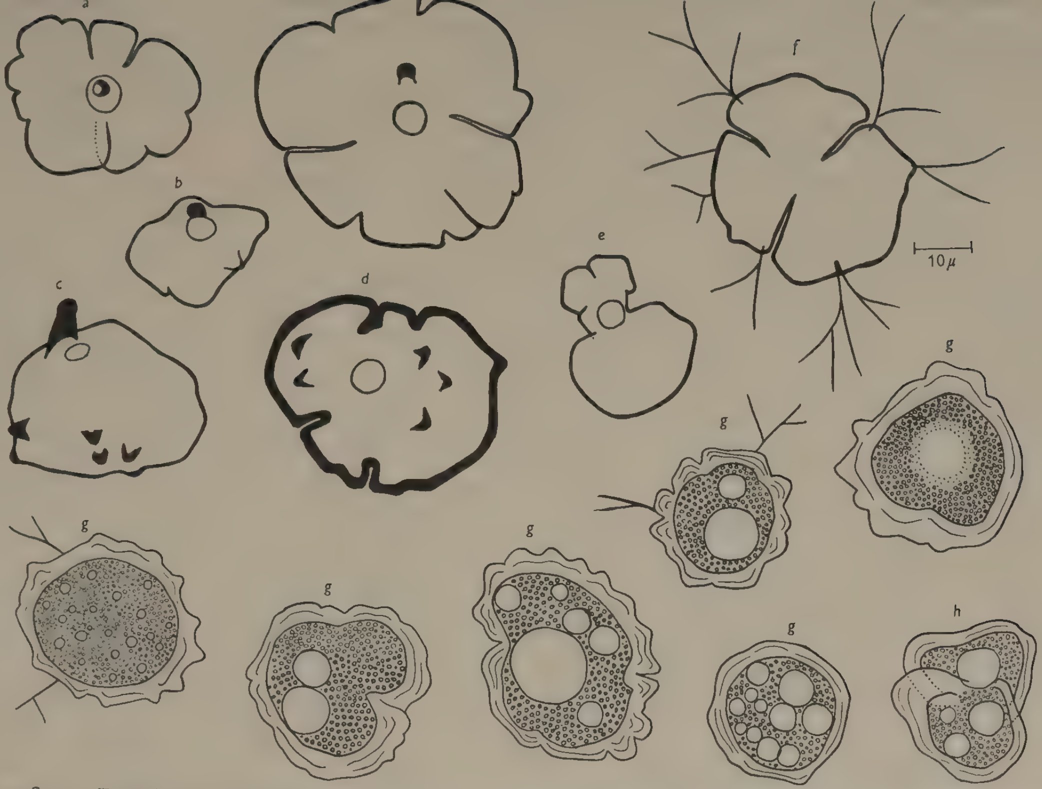
Entophlyctis lobata sp.nov.

This beautiful chytrid was obtained when permanently submerged mud from the littoral zone of Derwentwater was baited with purified shrimp exoskeleton (Karling, 1945). Although two other aquatic chitinophilic chytrids, namely *Asterophlyctis sarcoptoides* Petersen and *Chytriomyces hyalinus* Karling, were also present on the same substratum, there was little difficulty in distinguishing the species under discussion, which is pigmented and has a number of other distinctive characters.

The zoospore comes to rest and encysts on the surface of the substratum, and the sporangium rudiment and rhizoids are endobiotic (Text-fig. 1 *a*, *b*). The zoospore cyst usually becomes thickened (Text-fig. 1 *c*), and in consequence is persistent rather than evanescent. As the thallus grows larger its outline becomes distinctly lobed (Text-fig. 1 *c*–*h*; Pl. 13 *a*); eventually orange pigment appears and at this stage the region of clear cytoplasm which eventually emerges with the zoospores is recognizable, and characteristically the thickened persistent zoospore cyst is visible immediately above it (Text-fig. 1 *i*–*k*). At maturity the sporangium is often so deeply lobed that it appears to be constricted into a number of separate sectors (Text-fig. 1 *o*; Pl. 13 *b*. See also Text-fig. 2 *a*, *b*, *f*; Pl. 13 *c*, *d*). The rhizoidal axes, which are branched and extremely fine, are attached at a number of points (Text-fig. 2 *f*). The sporangium is normally flattened in the plane of the substratum, and the break-through to the surface by the exit tube occurs exactly perpendicular to this. An estimation of the precise length of the exit tube is therefore difficult, but since the sporangium is usually immediately below the zoospore cyst and not separated from it by a germ tube it must be extremely short in most cases. Even when a definite germ tube is present, there is no evidence that a lengthy exit tube is an associated feature (Text-fig. 2 *c*).



Text-fig. 1. *Entophlyctis lobata* sp.nov. *a*, Zoospores; *b*, young thalli with unthickened zoospore cyst (pigment derived from the zoospore globule is still visible at this stage); *c*, young thalli later with persistent zoospore cyst thickened; *d*, similar young thallus with germ-tube and thickened papillation; *e*, *f*, similar young thalli; *g*, an irregularly lobed thallus; *h*, vacuolate stage with thickened persistent zoospore cyst visible; *i*–*k*, thalli with evenly distributed orange pigment and central region of clear cytoplasm (*i* has a number of thickened papillations, but in *j* and *k* the single thickened knob in the centre is the persistent zoospore cyst); *l*, small mature sporangia; *m*, mature sporangium with no persistent zoospore cyst; *n*–*r*, mature sporangia, *n*, with thickened persistent zoospore cyst; *o*, *q*, *r*, with prominent papillations; *p*, with grossly thickened wall; *s*–*u*, portions of mature sporangia, *s*–*t*, showing relation of the persistent zoospore cyst to the substratum surface (cross-hatched); *u*, showing relation of the thickened persistent zoospore cyst to the region of clear cytoplasm and exit pore rim; *v*, dehiscing sporangium with zoospores in a ball at the mouth of the exit tube immediately before collective swarming (in *m*–*o*, *q*, *r*, *u* region of clear cytoplasm indicated by shading and the exit pore rim by the circle inside it; in *u*, figure top left, the inner circle represents the exit pore rim and the outer the position where the exit tube base has broken through the substratum surface). All drawings to the same scale.



Text-fig. 2. *Entophlyctis lobata* sp.nov. *a–f*, Dehisced sporangia; *a*, with thickened zoospore cyst apparently lying free in the exit pore; *b*, with thickened persistent zoospore cysts visible; *c*, with thickened persistent zoospore cyst and germ-tube, also thickened papillations; *d*, with thickened papillations; *e*, irregular dehisced sporangium; *f*, a deeply lobed one showing rhizoidal attachments on the undersurface; *g*, resting spores; *h*, deeply lobed resting spore. In *a–e* exit pore rim indicated by a circle. All drawings to the same scale.

The exit pore is some distance from the zoospore cyst in some instances (Text-fig. 2*b*); in others, however, it is partly blocked by the cyst, since this is apparently attached to its rim or close to it. However, the zoospores evidently emerge without hindrance (see mature and dehisced sporangia in Text-figs. 1*r*, *u*; 2*a*).

It is now necessary to consider certain dehisced sporangia which appear to be quite devoid of any persistent zoospore cyst (e.g. Text-fig. 2*e*), which might well have been present on the mature sporangium, only to be subsequently displaced and removed by the emerging zoospore mass. The dehisced sporangium in Text-fig. 2*a* indicates that this possibility is a real one. On the other hand, the fact that some mature sporangia also appear to be without a cyst (Text-fig. 1*m*) suggests other explanations. Either direct expansion from the zoospore cyst must have occurred, or development was indirect, with the zoospore cyst evanescent rather than persistent. On the whole, the latter explanation seems the more likely, since a recognizable cyst has been present on every young thallus which we have observed. A complicating factor in the recognition of the zoospore cyst is that certain thalli develop thickened papillations which are outwardly almost identical with it, both in size and shape (Text-figs. 1*i*, *o*, *q*, *r*; 2*d*). On these thalli, which incidentally are superficially like those of *A. sarcoptoides*, the thickened knob nearest the exit pore might represent either a persistent zoospore cyst or a thickened papillation, and in a late stage of development it is impossible to decide which is the correct interpretation.

Dehiscence is inoperculate and the zoospores form a ball at the mouth of the exit tube (Text-fig. 1*v*) and there undergo an unusual form of collective swarming. For some minutes the peripheral zoospores only are active, but finally the whole mass is involved and dispersal then proceeds rapidly.

The resting spores (Text-fig. 2*g*, *h*; Pl. 13 *e*) have a conspicuous thick yellow layered wall, the outline of which is usually irregular. In the material examined they were as numerous as the sporangia.

On the whole *Entophlyctis* seems the most appropriate genus for the Derwentwater chytrid, despite the fact that in the close proximity of the zoospore cyst to the exit pore there is a certain similarity with *Phlyctorhiza endogena* (Hanson, 1946). Since there is no described species to which it can readily be referred, a new one is proposed.

Entophlyctis lobata sp.nov. (Text-figs. 1, 2; Pl. 13).

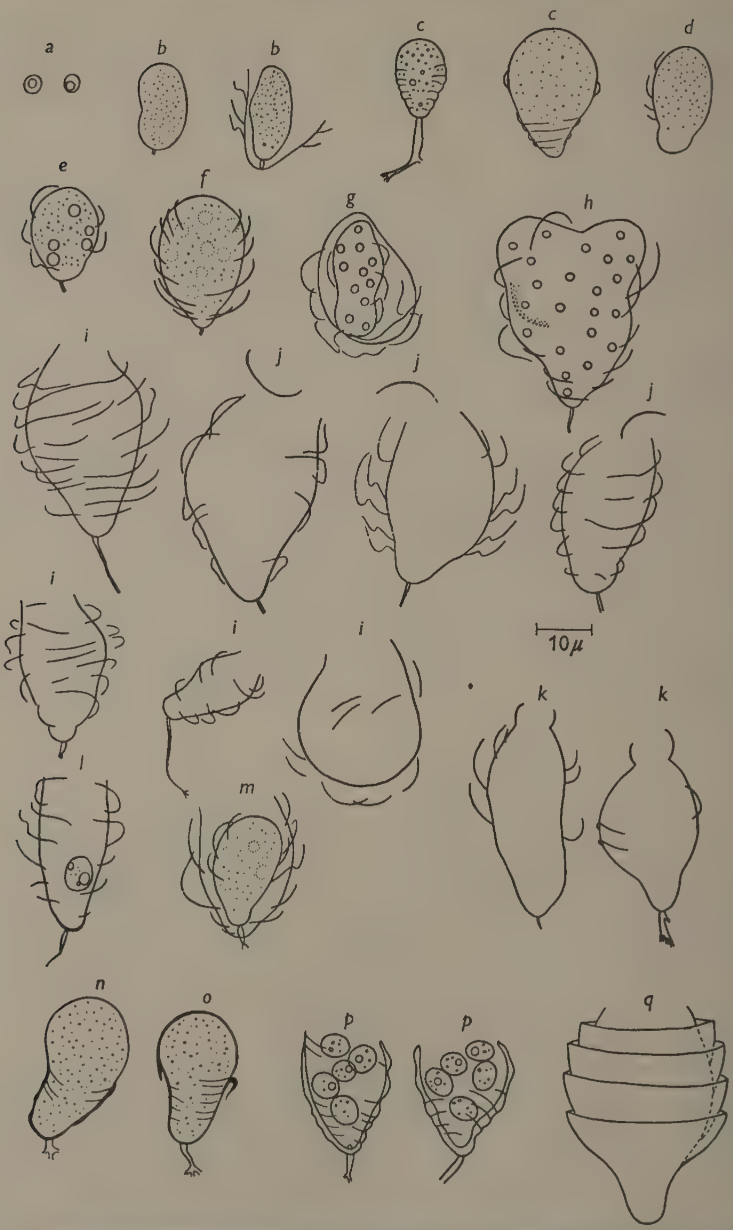
Thallus monocentricus, eucarpicus, consistens ex perstanti densato zoosporo epibiotico cum involucro, 2·2–3·5 μ lato, et endobiotico sporangio aut spora quiescenti cum nonnullis stirpibus ramosis et rhizoidalibus quae sunt distinctae et tenues. Adsunt aliquando sporangia tubulo unde crescunt germina densato. Adsunt quoque aliquando sporangia quae habent nec zoosporum cum involucro perstans nec tubulum unde crescunt germina. Sporangia forma globosa 12–50 μ lata, ovoidea 32–40 × 27–30 μ, aut oblonga 25–45 × 20–32 μ, complanata in plano substrati, cum muro 0·5–1·5 μ crasso. Alia sporangia sunt alte lobata, alia sunt papillis eminentibus, alia his ambobus proprietatibus sunt praediti. Papillae, quarum aliae sunt densatae, aliae non, sunt 2–4 μ latae et 5 μ tenus altae. Zona hyalinae materiei glutinosae quae excedit cum zoosporis lata est 10·5–13·5 μ, et foramen unicum 4·5–6·0 μ latum. Hoc foramen saepe proxime adiacet zoosporo cum involucro perstanti. Zoosporeae natando inter se glomerant antequam diffluunt. Zoosporeae dum movent sunt ovoideae, 3·5–4·0 × 2·5–3·0 μ, cum una olei guttula coloris aurantiae 1·0–1·5 μ lata, prope flagellum insertum sita. Flagellum posterius 15 μ est longum. Sporae quiescentes globosae sunt, 20–35 μ latae, aut ovoideae 27–40 × 20–30 μ, formae inaequalis, nonnunquam alte lobatae, cum flavo muro stratorum 2–6 μ, et una aut pluribus olei guttulis magnis (12·5 μ tenus latis) cum omnino sunt maturae. Ex luto submerso ex Derwentwater, Regione Lacuum Anglicorum, saprophyticus, esca 'chitin' fruens. Typus: Herb. I.M.I. 80976, legit 10 Mar. 1960.

Thallus monocentric, eucarpic, consisting of a persistent, thickened, epibiotic zoospore cyst, 2·2–3·5 μ diam., and an endobiotic sporangium or resting spore with a number of separate, delicate, branched rhizoidal axes. Sporangia with a thickened germ tube occur occasionally. Sporangia with no persistent zoospore cyst or germ tube occur occasionally. Sporangia spherical 12–50 μ diam., ovoid 32–40 × 27–30 μ, or oblong 25–45 × 20–32 μ, flattened in the plane of the substratum, and with a wall 0·5–1·5 μ thick. Sporangia are deeply lobed or with prominent papillations, or both. Papillations, which may or may not be thickened, are 2–4 μ wide and up to 5 μ high. The zone of clear gelatinous substance which emerges with the zoospores is 10·5–13·5 μ diam., and the single exit pore 4·5–6 μ diam. The exit pore is often very close to the persistent zoospore cyst. Zoospores undergo collective swarming before they disperse. Zoospores are oval in motion, 3·5–4 × 2·5–3 μ, with an orange oil globule 1–1·5 μ diam., situated near the flagellum insertion. Posterior flagellum 15 μ long. Resting spores spherical 20–35 μ diam., or ovoid 27–40 × 20–30 μ, irregular in outline, sometimes deeply lobed, with a yellow layered wall 2–6 μ thick, and one or more large (up to 12·5 μ diam.) oil globules at maturity. From submerged mud from Derwentwater, English Lake District, saprophytic on chitin bait. Type: Herb. I.M.I. 80976, coll. 10 Mar. 1960.

Chytriomyces poculatus sp.nov.

This species is characterized by a type of sporangium ornamentation which appears to be unique in the chytrids, consisting of a series of thin overlapping cupules of wall material. It has been obtained on keratin (snake skin) bait only, from soil samples collected at Shap and Naddle Forest (east of Haweswater) and at Haile (N.W. of Wastwater). All three samples were peaty and the pH of the liquid in the baiting dishes (5–5·6) was somewhat lower than that usually recorded when Lake District soil is investigated. *Septosperma rhizophidii* (Whiffen, 1942) was present at all three sites, and though our observations indicate that this parasitic chytrid is capable of attacking a number of saprophytic species, *C. poculatus* appeared to be immune, and it therefore seems possible that the cupule ornamentation may confer an advantage in this respect.

In the earliest stages of development the thallus wall is perfectly smooth (Text-fig. 3*b*). Very soon, however, delicate crenulations become visible (Text-fig. 3*c*), suggesting that cupule formation occurs as the result of fragmentation of the wall. Eventually vacuoles become visible in the cytoplasm (Text-fig. 3*f*) and from this stage onwards the cupules are a prominent feature. At maturity (Text-fig. 3*g*, *h*; Pl. 14 *b*) hyaline zoospore globules are visible, and after dehiscence a conspicuous detached operculum can often be seen (Text-fig. 3*j*; Pl. 14 *c*). The shape of some mature



Text-fig. 3. *Chytriomyces poculatus* sp.nov. *a*, Zoospores; *b–f*, young thalli, *b* with no wall ornamentation visible; *c*, showing crenulations of the wall; *f*, with vacuolate cytoplasm; *g*, a mature sporangium which has developed inside a dehisced one; *h*, a large mature sporangium; *i–m*, dehisced sporangia; *j*, showing detached opercula; *k*, an unusual form, with a constriction near the apex; *l*, *m*, with young thalli which have developed inside; *n*, *o*, young thalli with irregular wall thickening; *p*, dehisced thick-walled sporangia with young thalli inside; *q*, diagrammatic representation of wall ornamentation of overlapping cupules. Thalli *d–m* show typical wall ornamentation. All drawings to the same scale.

sporangia (e.g. Text-fig. 3*h*) suggests that two or more opercula occur occasionally, but it has not been possible to confirm this. Zoospores which fail to escape from the sporangium at dehiscence usually germinate *in situ* (Text-fig. 3*b*, *g*, *l*, *m*, *p*; Pl. 14 *d*).

In the material examined the only thalli which resembled resting spores were sporangia with heavily thickened walls (Text-fig. 3*p*). These, unlike more typical sporangia, completely lack projecting cupules, although in a certain stage of their development (Text-fig. 3*o*) there is often a peculiar wall outgrowth which is very difficult to interpret satisfactorily.

A number of monocentric eucarpic chytrids with sporangial ornamentation have been described (Sparrow, 1938), and these include species with projecting spines, hairs, lobes or teeth. However, there is no species with an ornamentation which is in any way similar to the peculiar frill-like envelopment described above.

Chytriomyces poculatus sp.nov. (Text-fig. 3; Pl. 14)

Thallus monocentricus, eucarpicus, consistens ex epibiotico sporangio et una rhizoido tenui. Sporangia ovoidea plerumque, 20–37 μ longa × 12–22 μ lata, aut ellipsoidea 17–37 μ longa × 9·5–14 μ lata. Sporangia etiam subglobosa aut piriforma raro inveniuntur. Superficies sporangii ornata est decem tenus tenuibus poculis, quorum aliud super aliud excedit. Haec a sporangio extenduntur 5·5 μ tenus. Rhizoidus est compositus stirpe 3–15 μ longa et 0·5–1·5 μ lata, cum ramis tenuibus in infima parte. Zoosporae liberantur cum operculum, quod formam tholi habet 5–13·5 μ latum, a summo sporangio seiungitur. Zoosporae sunt 3·5 μ latae, cum guttula olei hyalina 1·5 μ lata. Non observavimus quomodo se gerant zoosporae cum sporangium fit vacuum. Neque observavimus sporas requiescentes.

Ex humo complurium locorum in Regione Lacuum Anglicorum. Saprophyticus, esca 'keratin' fruens. Typus: Herb. I.M.I. 80977, legit 11 Dec. 1959.

Thallus monocentric, eucarpic, consisting of an epibiotic sporangium and a single delicate rhizoid. Sporangia mostly ovoid, 20–37 μ long × 12–22 μ wide, or ellipsoid 17–37 μ long × 9·5–14 μ wide. Subglobose or pyriform sporangia also occur rarely. The sporangium surface is ornamented with up to 10, thin, overlapping cupules of wall material. These extend up to 5·5 μ from the sporangium. The rhizoid consists of a stalk 3–15 μ long and 0·5–1·5 μ wide, with delicate branches at the base. Zoospores are released when a domed operculum, 5–13·5 μ diam., is detached from the sporangium apex. Zoospores are 3·5 μ diam., with a hyaline oil globule 1·5 μ diam. Behaviour of the zoospores at dehiscence not observed. Resting spores not observed.

From soil from several localities in the English Lake District. Saprophytic on keratin bait. Type: Herb. I.M.I. 80977 collected 11 Dec. 1959.

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EXPLANATION OF PLATES 13 AND 14

PLATE 13

Entophlyctis lobata sp.nov. *a*, Young thalli; *b*, large mature sporangium; *c*, deeply lobed dehisced sporangium with rhizoids visible; *d*, two thick-walled dehisced sporangia; *e*, resting spores (all × 850).

PLATE 14

Chytriomyces poculatus sp.nov. *a*, Young thalli with a dehisced sporangium below; *b*, large mature sporangium; *c*, dehisced sporangium with detached operculum; *d*, young thallus developing inside a dehisced sporangium; *e*, dehisced sporangium with young thalli below it (all × 900).

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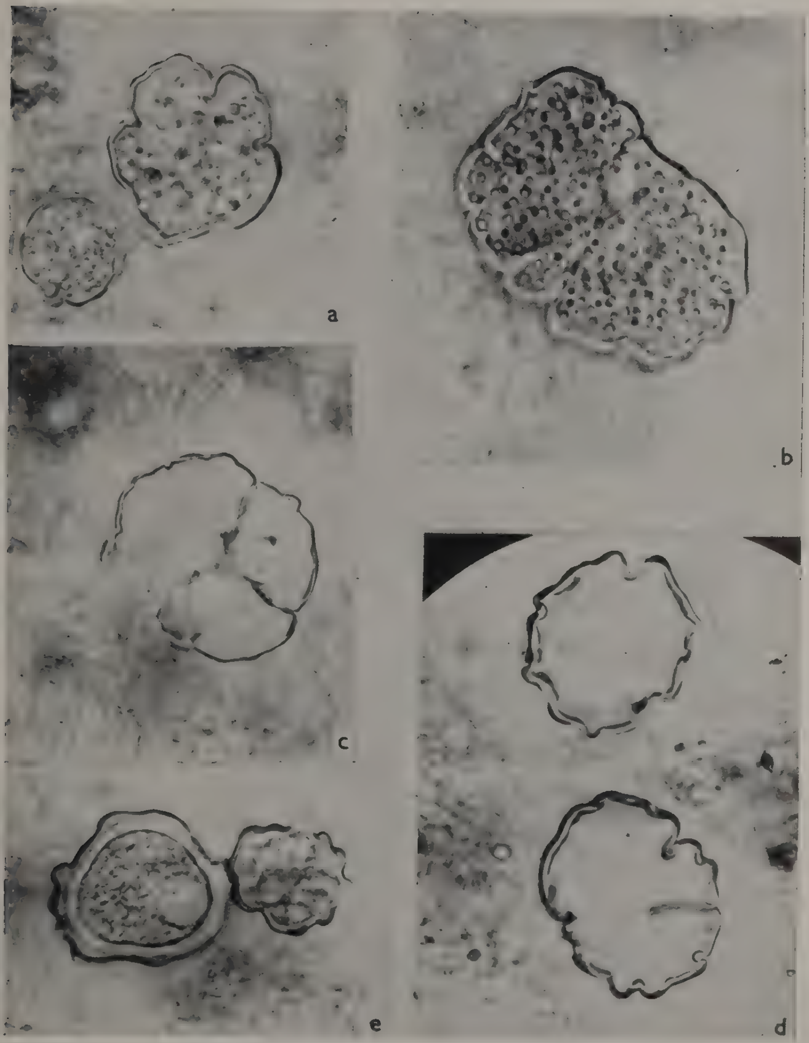

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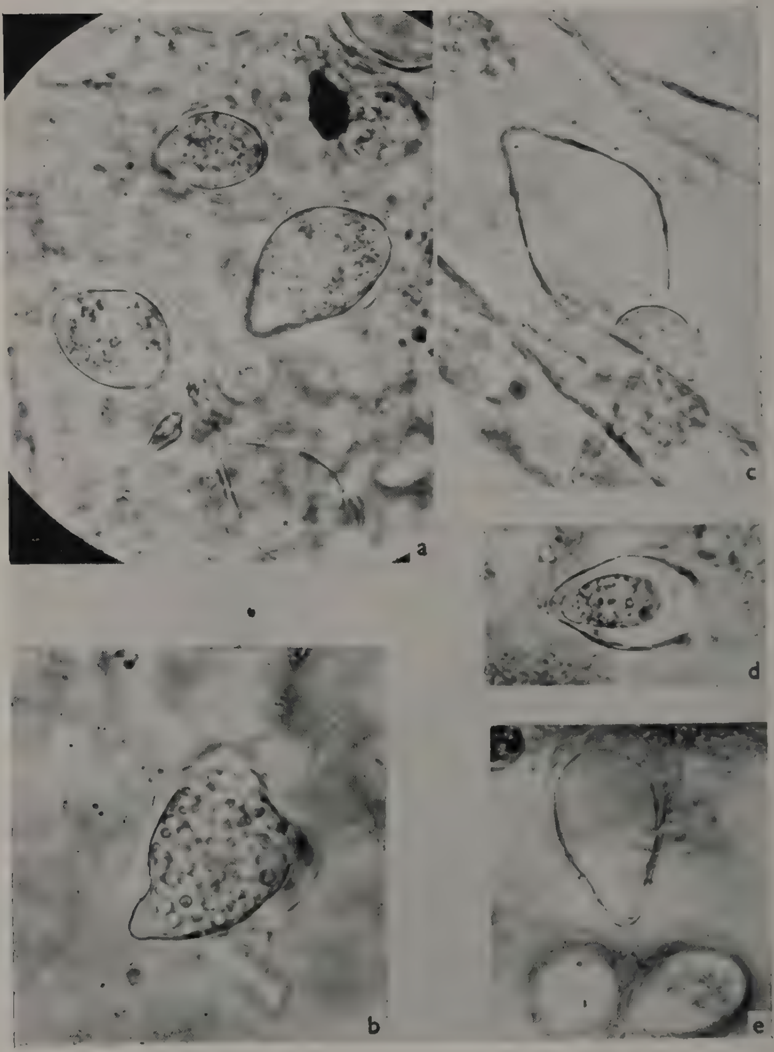
a
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MORPHOGENESIS IN AND THE EFFECT OF LIGHT ON *BLASTOCLADIELLA BRITANNICA* SP.NOV.*

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(With Plate 15 and 11 Text-figures)

The developmental history in pure culture of a single-spore isolate of a *Blastocladiella*, originally sent to us from Great Britain by L. G. Willoughby, has been investigated in some detail. Its wide range of morphological variability led to the isolation of several distinct substrains, one of which was incapable of producing resistant sporangia and another produced them in abundance. Formation of resistant sporangia was affected by several environmental factors, but most strikingly by white light. As a consequence of these and other observations, the fungus has been designated *B. britannica* sp.nov.

During an ecological investigation of the lower saprophytic fungi in Esthwaite Water in the English Lake District, Willoughby (1959) discovered what appeared to be a new species of *Blastocladiella* Matthews, the first member of this genus to be isolated in Great Britain. Subsequently, he graciously sent us a pure culture of his single zoospore isolate and we are very grateful to him for affording us the opportunity to study this most interesting organism further.

We shall present additional evidence which bears on the taxonomy of the genus and, in particular, data and observations supporting Willoughby's suggestion that we are dealing with a new *Blastocladiella*. Also, aside from morphological considerations, it became apparent that single spore isolates of *B. britannica* exhibited great variability in developmental potential. We shall describe some aspects of this variability, with particular emphasis upon the influence of temperature, nutrients and light.

MATERIALS AND METHODS

All pure cultures of *B. britannica* were derived from the original single spore isolate. Stock cultures were maintained on Difco CANTINO PYG AGAR (1·25 g. peptone, 1·25 g. yeast extract, 3 g. glucose, and 20 g agar/l. water; pH 6·8). Liquid cultures were grown on Difco CANTINO PYG BROTH containing bromcresol purple indicator. Broth cultures were held at constant pH by intermittent neutralization with NaOH. In most experiments with agar media the relatively small number of plants per unit volume precluded a significant change in pH, making the addition of bromcresol purple unnecessary. Agar plates were routinely inoculated as follows: a thin layer of surface agar (*ca.* 3–5 mm.^{2}) bearing a few dozen mature plants was placed in 8 ml. sterile distilled water at 22° C. A suspension of zoospores was obtained in 4–8 hr. and a few drops of this were distributed uniformly over the agar surface. Cultures were incubated at 22° unless otherwise specified.

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Dry weights were obtained after drying the plant material from liquid cultures at 90° to constant values. In all studies involving light (fluorescent; Sylvania, Standard, Cool White) the incident level was 180 f.c. at 22°; dark controls were always maintained at 22°. For lactic acid determinations the spent culture media were extracted with ether and the extracts analysed according to Ryan's (1958) microdiffusion method.

OBSERVATIONS AND EXPERIMENTAL

Conditions for optimal growth

Early studies revealed that growth was prolific over a broad pH plateau (6–8); therefore, subsequent studies were carried out at pH 6·8. The optimum temperature for growth was 16–25° (Text-fig. 1). Like so many of the water moulds (Cantino, 1955; Cantino & Turian, 1959), *B. britannica* is a vigorous lactic acid producer; cultures in liquid media must be neutralized periodically to insure a constant pH (Table 1). Furthermore, it is a

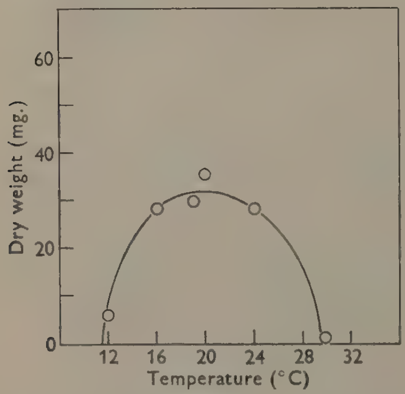


Fig. 1

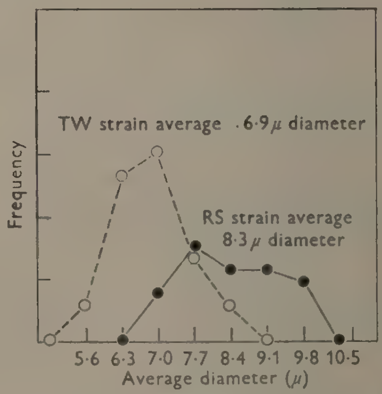


Fig. 2

Text-fig. 1. Representative temperature–response curve for PYG broth (100 ml.) culture inoculated with uniform spore suspensions and harvested after 8 days. Each point represents the average value from duplicate cultures.

Text-fig. 2. Distribution curves for zoospore diam. of TW strain and RS strain. (Fixed with 1 % osmic acid and stained with gentian violet.) Each division on the vertical axis represents 5 spores.

Table 1. *Lactic acid production by* B. britannica

Medium	1 l. PYG	1 l. PYG minus glucose
Wet wt.	19·1 g.	4·6 g.
Lactic acid recovered from spent medium	177·5 mg./l. or 9·3 mg./g. wet wt.	45 mg./l. or 9·8 mg./g. wet wt.

striking fact that the yield of lactic acid/unit weight of organism was the same, whether or not glucose was present in the medium, which suggests a strong tendency toward a fermentative mode of metabolism.

Morphological variability

Concurrent with the foregoing studies, the lineage of many isolates from subcultures of the original single spore isolate was followed through as many as fifteen generations, with environmental conditions maintained as constant as possible. As a result, it became clear that there was tremendous morphological variability, both quantitative and qualitative, whether populations were derived from a single plant or from a number of plants of the same morphological variety. The different types were as follows (Table 2):

Table 2. *Morphological types produced by* B. britannica*

	Progeny from		
	TW plants	RS colonies	TW colonies
% of trials in which RS plants appeared in 1st generation	40	65	15
% of trials in which RS colonies appeared on 1st generation plates	30	45	5
% of trials in which TW colonies appeared on 1st generation plates	40	55	85
% of trials in which TW plants appeared in 1st generation	95	75	100

* Tests were made at least in duplicate for every age category (2–6, 7–11, 12–16, 17–21, 22–26, 27–31 days), using cultures of different histories selected at random over 6 months.

Individuals with a resistant sporangium (RS plants), having the brown, pitted thick wall typical of this genus.

Individuals, thin-walled (TW plants), which liberated spores only when placed in water. They were a barely perceptible, light yellow colour, which was most easily seen when they were crowded together in the colonies described below.

RS colonies consisting of both RS plants and TW plants, derived from spores liberated from a TW plant which had discharged directly on its agar medium.

TW colonies consisting exclusively of TW plants, derived from spores liberated from a TW plant which had discharged directly on its agar medium.

Even within these four major groups morphological variants appeared; a few should be mentioned at this time though detailed studies remain to be done. For example, thalli of TW plants generally bore an extensive rhizoidal system causing such plants to have a very shaggy appearance ('shaggy' plants, Pl. 15 *g*); however, strains of TW plants were selected which bore a sparse rhizoidal system, giving them a relatively smooth appearance ('smooth' plants, Pl. 15 *f*). It should be emphasized that such strains do not simply represent the extremes of a morphological distribution curve, for intermediates were seldom seen. At the same time, we were able to select out two different RS-producing strains, one with RS averaging 98 × 101 μ, and the other 122 × 124 μ; again, few intermediates were ever seen.

Because of such clear-cut, inherent heterogeneity in *B. britannica*, certain strains were repeatedly and selectively subcultured until they 'bred true'; these were used in the following studies.

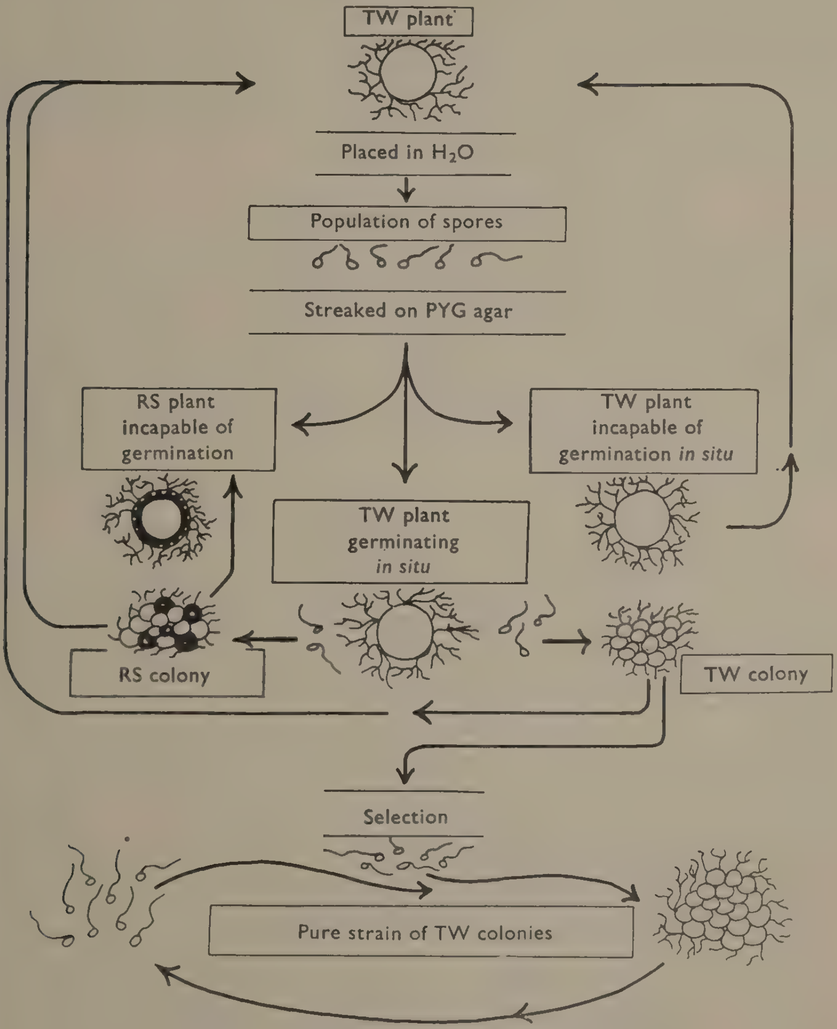
Pure strain of TW colonies

The data in Table 2 had revealed an obvious tendency for TW colonies, in contrast to TW plants and RS colonies, to yield an abnormally high percentage of TW colonies and a low percentage of RS plants and RS colonies upon successive subculturing. By repeated selection from TW colonies we succeeded in obtaining strains which produced *only TW colonies*! When solid media were inoculated with zoospores from such TW colonies, the viable, 1st-generation plants discharged on the agar to produce new colonies whose composition was identical with that of the parental TW colonies, i.e., RS plants were never formed. While their temperature range for growth was normal (Text-fig. 1), their generation time was *ca.* 5–6 days, compared with *ca.* 40 hr. for ordinary TW plants which give rise to RS colonies. Perhaps most surprising was the fact that the size of zoospores produced by the pure strain of TW plants was strikingly smaller than of those liberated by the parental RS strains (Text-fig. 2). TW colonies gradually developed a yellow pigmentation which reached maximum intensity at 22° when they were about 10 days old. The solubility and absorption spectrum (peaks at *ca.* 436, 463 and 492 mμ in petroleum ether) left little doubt that the material was a carotenoid, a fact quite consistent with the known distribution of such pigments in the Blastocladiaceae (Cantino & Hyatt, 1953*a*; Cantino & Horenstein, 1956; Emerson & Fox, 1940; Turian & Cantino, 1959).

Inasmuch as 1st-generation plants of *B. emersonii* Cantino & Hyatt could be induced to form resistant sporangia by addition of $NaHCO_3$ to PYG agar (cf. Cantino & Turian, 1959, for review), attempts were made to induce *B. britannica* to do the same. In spite of repeated trials with a variety of bicarbonate concentrations, the results were uniformly negative.

Then, because different concentrations of PYG had affected the incidence of resistant sporangia in the RS strain (see below), we grew cultures of the pure strain of TW colonies on serial dilutions of the basal PYG agar. Although the number of TW colonies increased as the concentration of PYG medium decreased (Text-fig. 4), neither RS plants nor RS colonies were ever produced. Similarly, modification of the glucose concentration in PYG (from 0·3 % to zero) did not induce RS formation.

Finally, because of the profound effects of light and dark upon the RS strain (see below), cultures of the pure strain of TW colonies on PYG were incubated in light and dark. The composition of populations grown in the light was: 40 % TW colonies, 17 % TW plants, and 43 % non-viable, compared with 10 %, 90 %, and nil, respectively, for those grown in the dark. Once again no RS plants were produced. Thus, we have concluded that our pure strain of TW colonies, incapable of producing RS plants even though it was derived from a parental culture which had this ability, had suffered a permanent metabolic lesion of some sort in its cytoplasmic and/or chromosomal machinery. We fully expect that it will serve as a valuable tool in our proposed studies on morphogeneis in *B. britannica*.


TW plant
Placed in H₂O
Population of spores
Streaked on PYG agar
RS plant incapable of germination
TW plant incapable of germination *in situ*
TW plant germinating *in situ*
RS colony
TW colony
Selection
Pure strain of TW colonies

Text-fig. 3. Developmental potentialities in *B. britannica*.

THE RS COLONIAL STRAIN

Life history

As was shown in Table 2, an assortment of progeny can be derived from an RS colony which is composed of varying proportions of RS plants and TW plants (Text-fig. 3). We would like to emphasize, at the start, that we have been totally unsuccessful in inducing even one RS plant to discharge spores. Endless attempts have been made, using plants of many ages (3 days to 5 months) grown under a wide variety of conditions and maintained on wet agar media, soil, desiccated filter-paper strips, and liquid cultures (nutrient media, soil media, hemp seeds in water, etc.). Furthermore, RS were subjected to all the usual treatments (heat shocks, cold treatments, wetting agents, fat solvents, nutrients of an almost infinite variety, etc.) plus many unusual treatments, but with a singular lack of success. Thus, in the sections which follow, any reference to spore discharge from an RS colony should be interpreted to mean that it is *only the TW plants* in such a colony which liberate spores.

Effect of temperature on RS plant formation

Our earliest observations with the original culture and its progeny suggested that temperature had a differential effect on RS formation. Subsequent experiments revealed that when spores derived from RS colonies were incubated on PYG agar at 12, 16, 19, 22, and 25° until all 1st-generation plants had reached maturity, individual RS plants were seldom produced at 12, 16 or 19°; but, at 22 and 25°, up to half of the total population consisted of RS plants! Clearly, RS formation was favoured by elevated temperatures.

Effect of bicarbonate on RS formation

Reasoning once again from our studies of the bicarbonate trigger mechanism in *B. emersonii*, various concentrations of $NaHCO_3$ were incorporated into PYG agar at pH 6·8; media were inoculated with *B. britannica*, and also with *B. emersonii* for comparison, and incubated for 10 days at 22°. The results revealed two important facts: first, that the tolerance to bicarbonate by *B. britannica* was quite different from that of *B. emersonii*; between 6×10^{-3} M and 2×10^{-2} M, the range within which *B. emersonii* grows well and is induced to produce RS plants, growth of *B. britannica* was inhibited completely; secondly, at 4×10^{-3} M bicarbonate and less, *B. britannica* did grow well, but RS formation was greatly delayed rather than promoted, in comparison with the time required for development in the absence of added bicarbonate. Finally, even total lack of atmospheric CO_2, which does affect the development of *B. emersonii* (Cantino & Horenstein, 1959), had no detectable effect on populations of *B. britannica*.

Just as with the pure strain of TW colonies, progressive dilution of the PYG medium caused a shift in the composition of the population of *B. britannica* (Text-fig. 5). But once again, when bicarbonate was incorporated in 1:10 PYG, the higher concentrations of bicarbonate indicated above inhibited growth, while at the lower concentrations RS formation was reduced relative to controls lacking bicarbonate. Thus, in its response to bicarbonate, *B. britannica* differs strikingly from *B. emersonii*, as Willoughby (1959) has suggested.

Effect of glucose on RS formation

Alteration of the concentration of glucose in PYG had a remarkable effect upon RS formation. The number of individual RS plants rose from 26 % at 2 × normal glucose to *ca.* 90 % at nil; conversely, individual TW plants dropped from 56 % to *ca.* 10 %. The usually small number of colonies remained roughly constant (Text-fig. 6). On the other hand, alteration of

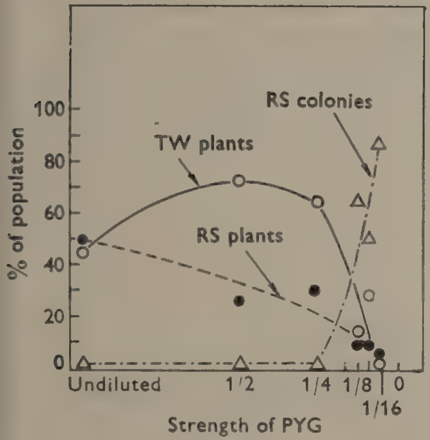


Fig. 4

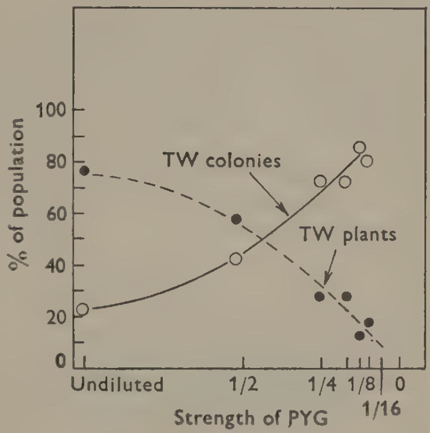


Fig. 5

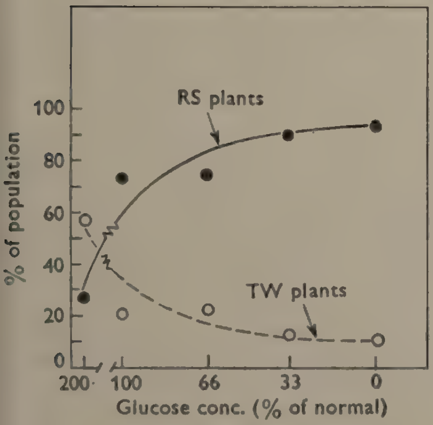


Fig. 6

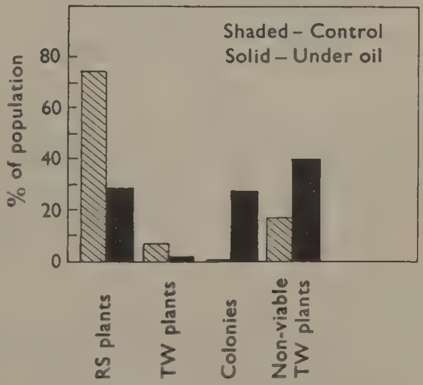


Fig. 7

Text-fig. 4. Effect of different concns. of PYG (2 % agar) on the composition of populations derived from spores of TW colonies.

Text-fig. 5. Effect of different concns. of PYG (2 % agar) on the composition of populations derived from spores of RS colonies.

Text-fig. 6. Effect of different concns. of glucose (in PYG) on RS formation. The low incidence of colonies, which was not plotted, remained approximately constant at all dilutions.

Text-fig. 7. Effects of semi-anaerobic conditions on the composition of populations derived from spores of RS colonies; 24 hr. after inoculation plates of PYG were covered with mineral oil 5 mm. deep and incubated for 5 days.

the peptone and/or yeast in PYG had no pronounced effect on the incidence of RS plants.

Because of the effect of glucose cultures were grown under reduced oxygen tensions. For example, under a flowing stream of nitrogen (p.p. of O_2, *ca.* 0·1 mm. Hg) *B. britannica* exhibited no detectable growth. Under somewhat higher partial pressures of O_2 (e.g. under mineral oil), growth did occur, but (relative to aerobic controls) there were more non-viable plants, individual RS plants decreased, and RS colonies increased (Text-fig. 7).

Effect of light on RS formation

Finally, because visible light accelerated RS formation in *B. emersonii* (Cantino, 1957) when bicarbonate was present, *B. britannica* was grown in the presence and absence of light. Text-fig. 8 illustrates the dramatic effect obtained. Consequently, the question immediately arose: throughout what period in ontogeny was light needed for formation of TW plants (or, conversely, inhibition of RS formation)? The results (Text-fig. 9) of an experiment designed to test this revealed that plants could be exposed to light for about 27 hr. and still retain their capacity for developing into RS when placed in the dark. Beyond this 'point of no return' a change in the light regime had no effect.

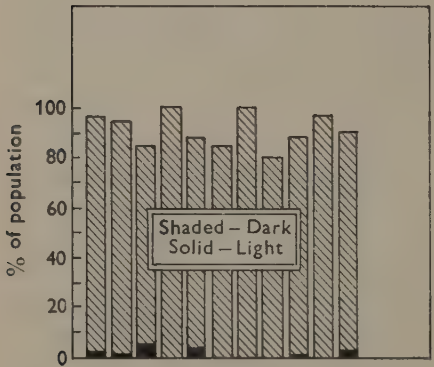


Fig. 8

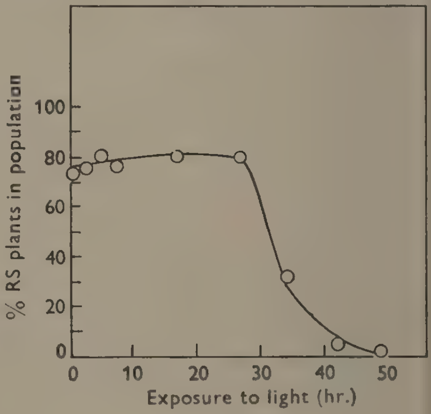


Fig. 9

Text-fig. 8. Effect of light on RS plant development. Inoculated plates of PYG were incubated in the light (or dark) for 3 days. Each bar represents the percentage of RS plants in a population derived from spores of a single RS colony.

Text-fig. 9. The relation between duration of light and RS formation. Two inoculated PYG plates were incubated in the dark for the entire growth period of 50 hr.; all others were exposed to 180 f.c. of white light for different times and then covered with aluminium foil for the remainder of the growth period. Each point is an average value for duplicate cultures.

Isolation of RS strains yielding 100 % *individual RS plants*

As was mentioned earlier, all attempts to effect germination of mature RS plants have been totally frustrated. On the other hand, we have been able to induce spore discharge in 'young', potential RS plants; the methods we have evolved to accomplish this should be very useful in experimental studies of *B. britannica*, in particular, and they may be applicable to other members of the Blastocladiaceae in general. We have already stressed that the usual population of mature plants on PYG agar plates is a heterogeneous and variable one. However, when this population is still young, i.e. 30–40 hr. old at 22°, all plants have essentially the same appearance, and it is possible to induce spore cleavage and discharge in many of them if they are simply removed from the agar and placed in water. Thus, by isolating individual thalli in this age group, inducing spore discharge, and inoculating these spores on to PYG agar plates, we found that the population which resulted was, when mature, usually as variable as the parent population. During the course of many such experiments, however, the population derived from the spores of such isolated plants occasionally consisted of a very high percentage of RS plants; this suggested to us that the young, isolated plants from which these spores had come may well have been potential RS plants, and that they would have developed thus, had they been permitted to reach maturity. Working on the assumption that we might be able to obtain a pure RS strain (populations of 100% *individual RS plants*) if we could select the right plants, this approach was continued on an expanded scale.

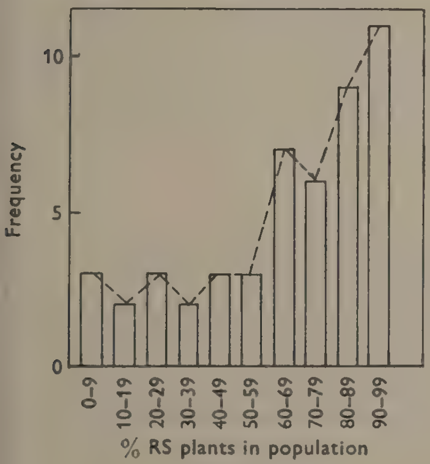


Fig. 10

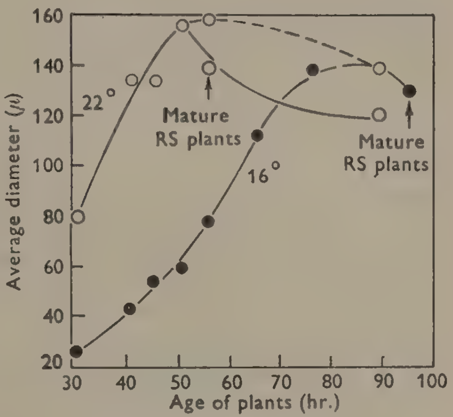


Fig. 11

Text-fig. 10. Results of attempts to select populations of vigorous RS producers. Each population counted consisted of progeny from a single sporangium.

Text-fig. 11. Growth curves of individual RS plants. Each point represents the average diam. of 20 plants. Beginning at *ca.* 50 hr. (22°), the RS wall (solid line) begins to shrink inward and away from the original thallus wall (dotted line).

Many 35 hr. old plants were isolated at random, placed individually into water in depression slides, and the discharged spores inoculated on to PYG agar. Then, when these 1st-generation populations were 35 hr. old, 5–10 individual plants were again isolated from each plate, and the process was repeated. And indeed, populations were finally obtained which consisted of 100 % RS individuals! Tabulation of the progeny derived from several individual plants (Text-fig. 10) revealed that we were effectively selecting from one end of the distribution curve. Typical growth curves at 16° and 22° for such RS strains, which produced only individual RS plants, are shown in Text-fig. 11. The maintenance of these strains presented a serious problem, because populations had to be subcultured every 35 hr., i.e. before they developed into mature RS which, as we pointed out, have defied all attempts to germinate them. However, the difficulty was partially circumvented by maintaining populations of these potential RS plants in an immature state for several weeks through the simple expedient of incubating them at 2°. Thus, plants were isolated from the 2° populations whenever they were needed for initiation of new subcultures of the pure strain of individual RS plants.

At this time we cannot say whether or not the swarmers derived from young, potential RS plants are the same, genotypically or phenotypically, as those that would have emerged from the same plant in its 'fully mature' state, that is, a resistant sporangium. Until we can devise the means for germinating RS plants, the question will remain unresolved, but we trust that soon the answer will be forthcoming.

Discussion

Morphological variability in Blastocladiella britannica

For experimental studies in morphogenesis, we could not have been provided with a more handsome little organism than *B. britannica*. No other species in the Blastocladiaceae which has been subjected to rigorous examination displays such a high degree of morphological variability—a variability that presumably is under phenotypic control (*sensu* Emerson, 1950). Three principal observations lead us to this presumption: first, we have never detected true, gametic copulation, thus probably precluding segregation of causative factors via meiosis; secondly, and perhaps more convincing, is the fact that a population of uninucleate spores, each originating from a single plant (which, in turn, is derived from a single, uninucleate spore), can exhibit a wide gamut of potential diversity; and thirdly, this developmental potential is strikingly modified by environmental conditions such as nutrients, temperature and light.

It seems almost axiomatic, therefore, that either organized control centres or other less specialized devices must carry this information, and that they are seated in the cytoplasm of the cell; furthermore, it seems to us a good guess that, following the final cleavage of the protoplast into thousands of spores, this machinery is distributed in random fashion resulting in a spore population which varies over a wide range in its content of these control centres. The data in Text-fig. 10 lend credence to this hypothesis, for they clearly illustrate that we were selecting plants from that portion of the population in which these control devices, whatever their exact nature, were present in either a very high or a very low concentration; selection of the correct alternative would be contingent on whether the potentiality for formation of resistant sporangia is associated with a relative abundance or relative lack of these hypothetical centres.

Finally, if this concept is tentatively accepted, then it would follow that the activity and/or reproduction rate of these control centres could be influenced by external conditions, and the most impressive effect so far demonstrable would be that exerted by light (as depicted in Text-fig. 8). In the latter experiment, for example, the population possessed a high capacity for formation of RS plants in the dark; this capacity was almost completely nullified by exposure to light.

At the moment, this hypothesis is founded on tenuous correlations and indirect evidence, but ample precedence has accumulated for assigning such vital, regulatory powers to the cytoplasmic components of the cells in the Blastocladiaceae. In *B. emersonii*, *Allomyces arbuscula* Butler, and *A. macrogynus* Emerson & Wilson (Cantino & Turian, 1959, discussion), the distribution of cytoplasmic, Nadi-positive, mitochondria-like particles, whose concentration in the cell is subject to environmental modification, has been correlated with biosynthesis of gamma carotene and, concomitantly, the capacity for morphological and sexual differentiation. Further experimental work with *B. britannica* should provide evidence as to whether or not it will fall into the pattern currently associated with its near relatives.

The taxonomic position of Blastocladiella britannica

Willoughby (1959) arrived at the tentative conclusion that his isolate of *Blastocladiella*, on general morphological grounds, was more closely related to *B. simplex* Matthews (1937) and *B. laevisperma* Couch & Whiffen (1942) than to other species of *Blastocladiella* (cf. Sparrow, 1960) but that, on the basis of spore size it more nearly resembled *B. emersonii* (Cantino & Hyatt, 1953*b*). With Willoughby's observations and our own experiments in mind, we agree that most other described species of *Blastocladiella* are sufficiently different as not to merit further attention for taxonomic purposes; but *B. stuebenii* Couch & Whiffen (Stüben, 1939; Couch & Whiffen, 1942), whose superficial morphology is similar to that of *B. britannica*, does deserve consideration.

Inasmuch as we have assumed the responsibility for proclaiming *B. britannica* a new species, we feel compelled to testify in favour of its well-defined character on the basis of the additional parameters that have resulted from our work; thus, we will use Willoughby's (1959) conclusions as a convenient point of departure.

First of all, we should like to provide further, conclusive evidence that an unmistakable distinction exists between *B. emersonii* and *B. simplex*, on the one hand, and *B. britannica* on the other.

(*a*) When grown submerged in liquid culture or beneath the surface of solid media (PYG, 22°) both *B. emersonii* and *B. simplex always* produce long, cylindrical basal cells bearing a terminal rhizoidal system (Pl. 15*a*). Furthermore, even on the surface of PYG agar, shorter but distinct stalks are also *always* produced. Under indentical conditions *B. britannica never* produces a detectable stalk or basal cell; instead, in the fully grown plant, the rhizoids are attached at numerous points on the spherical thallus (Pl. 15 *b*). We believe that these comparative observations alone, made under rigidly controlled and reproducible conditions, provide sufficient morphological evidence that *B. britannica* is not identical with *B. emersonii* or *B. simplex*.

(*b*) In addition, under these same laboratory conditions, the resistant sporangia of *B. emersonii* and *B. simplex* germinate with ease; those of *B. britannica* do not.

(*c*) Finally, and again under controlled laboratory conditions where populations have ample 'lebensraum', both *B. emersonii* and *B. simplex* always liberate spores via pores, each resulting from deliquescence of a papilla, while *B. britannica* always does so through obvious exit tubes of different lengths (Pl. 15 *c*).

Having eliminated *B. emersonii* and *B. simplex* from consideration, there remain only *B. laevisperma* and *B. stuebenii*. Here, we have had to resort to a comparison of our observations of *B. britannica* with the data available in the literature about *B. laevisperma* and *B. stuebenii*, as follows:

(*a*) The living spores of *B. britannica* average 4·5–6 × 7·5–11 μ (Willoughby, 1959); fixed preparations are not significantly different (av. 7·3 × 9·4 μ). Even the spores of the substrain of TW colonies, which are distinctly smaller than those of the parental 'wild type', measure 6 × 7·7 μ. On the other hand, the spores of *B. laevisperma* (Couch & Whiffen, 1942) are 3·8–4·6 × 6–6·5 μ, and those of *B. stuebenii* (Stüben, 1939) average 3·5 × 4·8 μ.

(*b*) The resistant sporangia of *B. laevisperma* (Couch & Whiffen, 1942) and *B. stuebenii* (Stüben, 1939) have no pits; those of *B. britannica* always bear very small pits (*ca.* 1 μ diam.; cf. Pl. 15 *d*, *e*).

(*c*) The resistant sporangia of *B. laevisperma* are reported to germinate after a short 'rest period' (Couch & Whiffen, 1942). The resistant sporangia of *B. stuebenii*, when grown on dilute peptone media (Stüben, 1939), whereon the thickness of the wall was minimized, also germinated with regularity. The resistant sporangia of *B. britannica* are not produced on such dilute peptone media. But, under the various conditions under which we have induced resistant sporangia to form, germination was never obtained.

Thus, *B. laevisperma* must be eliminated from consideration.

However, in its spherical nature, its profuse rhizoidal system, and its development from an enlarged reproductive rudiment derived from the swollen body of a spore, *B. stuebenii* does display some characteristics in common with *B. britannica*. A few more distinguishing parameters of a general physiological nature, therefore, will help to rule it out of the picture. All references to *B. stuebenii* are taken from Stüben (1939).

(*a*) The optimum temperature for growth of *B. stuebenii* on peptone (and other) media is 34°, whereas that for *B. britannica* is 16–26° on peptone media; indeed, growth does not occur above 32° under any condition.

(*b*) Under crowded conditions on agar media, *B. stuebenii* produces colonies in which plants tend to become distinctly elongated; furthermore, in such crowded conditions, resistant sporangia are produced in the centres of the densely populated colonies while thin-walled plants are produced on the periphery; *B. britannica*, under these sorts of conditions, behaves in diametrically opposite fashion; the thalli never elongate, resistant sporangia are produced round the edges of thickly populated colonies, and thin-walled plants are produced in the centre.

(*c*) Finally, colonies of *B. britannica* on peptone media gradually accumulate an unmistakable yellow colour, due to the synthesis of carotene; Stüben never reported the appearance of such a pigment in colonies of *B. stuebenii.*

We feel justified, therefore, in designating Willoughby's (1959) isolate a new species.

Blastocladiella britannica sp.nov.

Thallus sporangium parietibus tenuibus colore subflavo (carotene), aut parietibus crassis brunneum puteis praeditum perdurans. In cultura liquida vel solida et nutriente sporangium semper sphaericum, sine stipite et cum patente compositione rhizoidali in locis multis thalli surgente. In officina in cultura pura thalli sporangiales perdurantes 98–140 μ diametro, pariete 4·2 μ. Genesis sporangiorum perdurantium per $NaHCO_3$ non creantur. Germinatio sporangiorum perdurantium et sexus non cognoscitur. Thalli parietibus tenuibus 80–180 μ diametro sporas posterius uniflagellatas, typice blastocladeaceas, 7·3 × 9·4 μ, per paucos tubos emittunt. Flagellum 20 μ longitudine. In basi solida progenies ex sporis maxime varia potestate crescendi, alios thallos parietibus tenuibus qui sporas in situ generant, alios thallos parietibus tenuibus qui sporas solum in aqua generant, alios thallos sporangiales perdurantes ferens. Evolutio praeter morem inclinata ad effectum lucis manifestae. Luce absente omnes fere sporae thallos sporangiales perdurantes generant, neque thallos parietibus tenuibus.

Collata et discreta per L. G. Willoughby ex Aqua Esthwaitensi (Regio Lacuum), Angliae, 1958; typus est Herb I.M.I. No. 84399.

Thallus a thin-walled sporangium with light yellow pigmentation (carotene) or a thick-walled, brown, minutely pitted, resistant sporangium. In liquid or solid nutrient media, always spherical, without stalk, and with extensive rhizoidal system arising at many points on thallus. Under controlled conditions in pure culture, resistant sporangial plants 98–140 μ diam., with wall 4·2 μ thick. Genesis of resistant sporangia not induced by bicarbonate. Germination of resistant sporangia and sexuality not known. Thin-walled plants 80–180 μ diam., liberating posteriorly uniflagellate, typically blastocladiaceous spores, 7·3 × 9·4 μ, through several tubes. Flagellum 20 μ long. On solid substrata, progeny from spores extremely variable in developmental potential, yielding thin-walled plants liberating spores *in situ*, thin-walled plants liberating spores only when placed in water, and resistant sporangial plants. Development extraordinarily susceptible to influence of visible light. In absence of light, almost all spores produce resistant sporangial plants instead of thin-walled plants.

Type. Collected and isolated by L. G. Willoughby from Esthwaite Water (Lake District), Great Britain, 1958. Herb. I.M.I. No. 84399 (slide and preserved material).

This research was supported by grants from the National Science Foundation and the National Institutes of Health to the second author. Thanks are also due to Professor William M. Seaman who kindly provided the Latin translation for our diagnosis.

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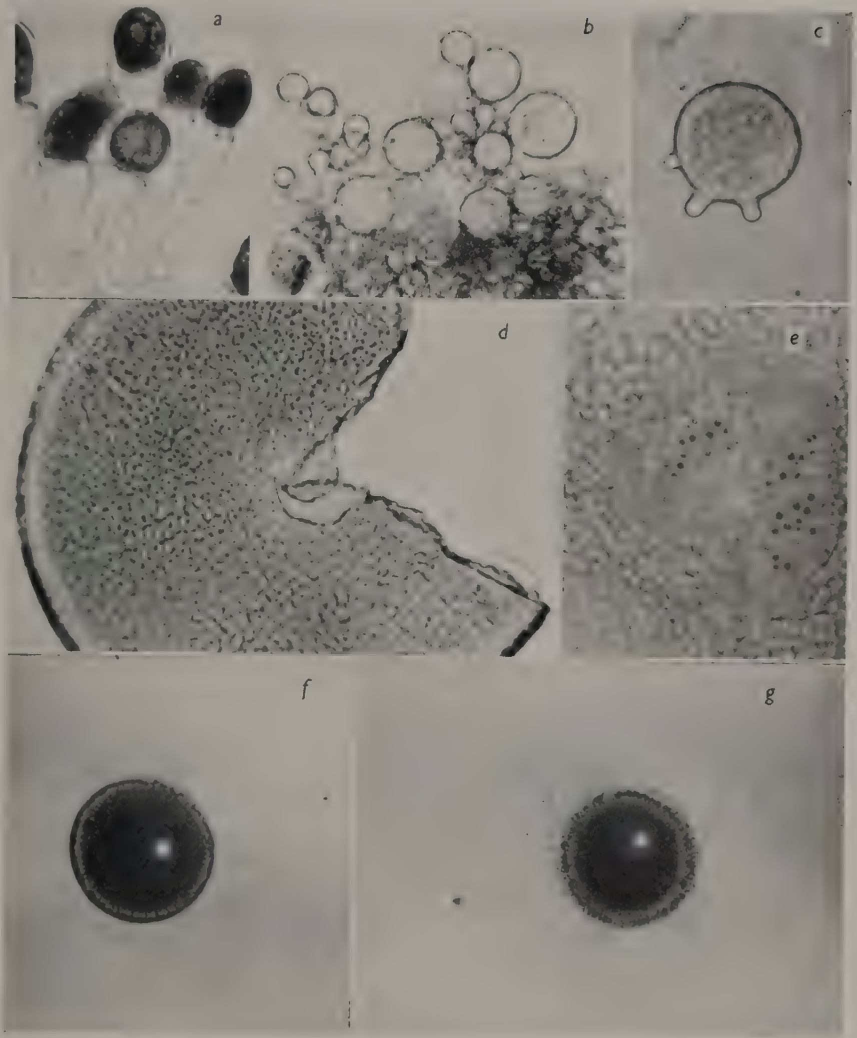
EXPLANATION OF PLATE 15

Blastocladiella britannica

a. Plants of *B. emersonii* grown in liquid media; note typical, elongated basal stalks. × *ca*. 55.
b. Plants of *B. britannica* grown in liquid media; note lack of stalks. × *ca*. 55.
c. Mature plants of *B. britannica* with developing discharge tubes. × *ca*. 115.
d. Resistant spoorangium of *B. britannica*; ruptured to illustrate pitted surface. × *ca*. 560.
e. Portion of D, enlarged. × *ca*. 1290.
f. 'Smooth' TW plant of *B. britannica*. × *ca*. 115.
g. 'Shaggy' TW plant of *B. britannica*. × *ca*. 115.

(*Accepted for publication* 19 *March* 1960)



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Trans. Brit. mycol. Soc. **44** (2), 199–213 (1961).

EFFECT OF LIGHT AND TEMPERATURE ON GROWTH AND DEVELOPMENT IN *THAMNIDIUM ELEGANS* LINK

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Botany School, University of Cambridge

(With Plate 16 and 7 Text-figures)

Thamnidium elegans produces either sporangia or sporangioles on discrete phototropic sporangiophores. The development of the sporangia resembles that of *Phycomyces*. The effects of light and temperature on the morphogenesis of the fungus have been studied by means of a specially built time-lapse camera.

Illumination has been shown to favour the production of sporangia, to alter the duration of Stage I elongation, and to cause a transient growth rate decrease in both Stage I and Stage IV sporangiophores.

The over-all rate of the reactions leading to the light-induced, growth-rate minimum shows a straight-line Arrhenius relationship with temperature, the growth rate minimum occurring earlier in Stage IV. The dark growth rates of Stage I and Stage IV are not affected by temperature in the same way. Experiments to show the relationship between the growth rate decrease and the positive phototropism are discussed, and it is suggested that the phototropic response falls into two well-defined stages.

In 1957, Hawker reported that the sporangia of *Thamnidium elegans* Link were only produced in the presence of light, and as taxonomic work was then in progress on other members of the Thamnidiaceae (Lythgoe, 1958) it was of interest to find out whether these other closely related fungi showed similar effects of light.

The sporangia of *Helicostylum elegans* Corda, *H. fresenii* (Van Tiegh. & le Monnier) Lythgoe and *H. venustellum* Lythgoe all proved to be much more numerous in cultures that had been illuminated. It was therefore decided to make a more detailed study of the effects of light on the morphogenesis of one of the members of the Thamnidiaceae. *Thamnidium elegans* was chosen for study chiefly because sporangia and sporangioles are produced in more or less equal numbers under ordinary laboratory conditions, and also because the sporangia and sporangioles are borne separately on well separated sporangiophores during the early stages in growth of the colony. There is also very little sterile aerial mycelium. This species is further suited for investigation in being stable in culture (Barnes, 1934) and in having simple nutritional requirements (Hesseltine & Anderson, 1956).

T. elegans was first described by Link (1809) and is the type species for the genus *Thamnidium*, which in turn is the type genus for the Thamnidiaceae. *T. elegans* is not commonly found in nature, and together with *H. fresenii* is usually found on cold-stored carcasses. A full account of the genus *Thamnidium* is given by Hesseltine & Anderson (1956).

Time-lapse photographs have shown that the sporangia of *T. elegans* develop in the same way as those of *Phycomyces*, and for this reason Castle's (1942) classification of the different stages of sporangial growth, which is adapted from that of Errera (1884), will be followed. Castle has divided the growth of the *Phycomyces* sporangiophore into four stages, as follows. Stage I: upward growth of the hypha which will ultimately produce a terminal sporangium. Stage II: the hypha has stopped growing and the terminal sporangium is produced and is increasing in volume. Stage III: no apparent growth in the sporangium and it is presumably maturing. Stage IV: the subsporangial region of the sporangiophore begins to elongate. During the first part of this growth there is a dextral twisting of the sporangiophore and this is Stage IV*a*; this rotation should then reverse to sinistral and this marks the beginning of Stage IV*b*. This reversal, however, has not been observed in *T. elegans*.

Plate 16 shows the behaviour of *T. elegans* grown at 17° C. throughout, with the experimental light switched on after 80 min. In this series of photographs taken at 40 min. intervals, Stages I–IV of sporangial development are clearly seen, but the droplets which are often produced by the Stage IV sporangium are not to be observed. It is these droplets which act as natural markers demonstrating the rotation of the sporangiophore. The droplets appear early in Stage IV and they persist for only about 2 hr.; perhaps for this reason the reversal of rotation marking Stage IV*a*–IV*b* has not been observed, and the presence of Stage IV*b* cannot be considered established. The sporangioles, on the other hand, do not produce droplets, and in no instance has any elongation corresponding to the Stage IV elongation of the sporangiophore been seen. In his original description, Link (1809) described and figured the fungus as having lateral tufts of sporangioles springing from the main sporangiophore, a feature now considered typical for the family (Hesseltine & Anderson, 1956). This condition is late in appearing in the colony; the tufts of sporangioles originate near the base of the elongated (Stage IV) sporangiophore.

Investigations were made to find the most suitable humidity and temperature at which to grow the fungus for the series of experiments on the effects of light. Low temperatures were found to favour the production of sporangia even in the dark as reported for *T. elegans* by Ribaldi & Canterelli (1953). Relative humidities that were only just high enough to allow fruiting bodies to develop also favoured the production of sporangia, but sporangia were produced only by cultures which had been previously illuminated. A temperature of 22·5° C. was found convenient and suitable for most of the experiments described below, and the fungus was grown over a saturated solution of potassium dihydrogen phosphate, which gives a relative humidity of about 98 % at 22·5° C. The isolate of *T. elegans* used in all these experiments was originally obtained by the late Professor F. T. Brooks in 1921 (Bot. Sch. culture M). The stock culture is maintained on malt agar. The growth medium used for experimental work is a synthetic one described by Hesseltine (1954) as being suitable for growing *Mucor*, and this medium is hereafter referred to as 'S.M.A.'

The apparatus used for this investigation is described in some detail, because the camera that was adapted to take time-lapse photographs that can be shown through a ciné projector is still freely and cheaply available on the 'surplus' market, and the adaptation does not require highly specialized workshop facilities.

METHOD

The apparatus consists of a fixed, time-lapse camera which photographs the developing fruit-bodies of *T. elegans* growing in a movable incubator, in which the humidity and illumination are controlled (Text-fig. 1). Every minute a photograph is taken, using a 10 sec. light flash of wavelength > 600 mμ.; no typical light-induced effects have been observed from this red photographic light alone. As both the camera and the experimental light source are fixed, the object in focus remains in a constant position with relation to the light source.

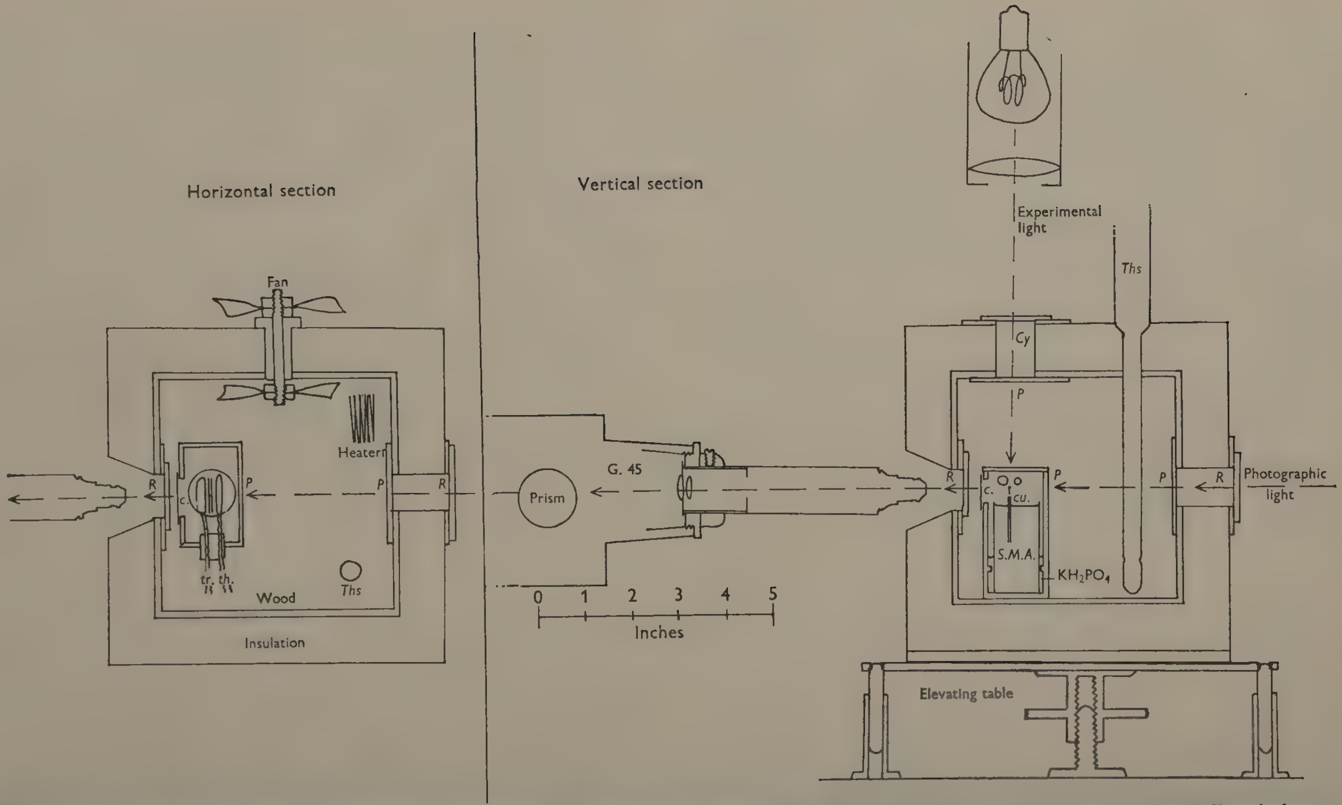
Method of culture. A sandwich of S.M.A. about 1 mm. thick is made by pouring a drop of molten medium on to a sterile square coverslip and placing another coverslip on top. When the agar has set, the sandwich is set upright in a specimen tube full to the brim with solid S.M.A. The middle of the top edge of the sandwich is lightly inoculated with a stab. The tube is then placed in a larger specimen tube which is plugged with cotton-wool and kept in the dark at 22·5 C. for about 30 hr., after which it is transferred to the humidity chamber next to be described, care being taken to exclude light during the transfer. Under these conditions spikes begin to appear after a further 12 hr.

The humidity chamber. The humidity chamber consists of a Perspex box fitted with a removable lid. The culture tube is loosely fitted into a round hole in a false floor $\frac{1}{2}$ in. above the base. A saturated solution of potassium dihydrogen phosphate, giving a relative humidity of about 98 % at 22° C., fills the bottom of the box and is deep enough to cover the false floor. One side wall is bored with a $\frac{1}{2}$ in. diam. hole to take the temperature and light measuring instruments. A $\frac{3}{4}$ in. diam. hole is bored in the face towards the camera, and a coverslip is stuck over the hole with 'Vaseline' to act as an optical window. All surfaces except the lid and the side facing the photographic light are painted black.

The incubator. The incubator is a plywood box with a removable lid. All faces except the base are covered with a 1 in. thick layer of 'Polyzote', an exceedingly efficient expanded plastic insulator. The base is bolted to a flat brass plate. The box is painted black to reduce light scatter inside and the external 'Polyzote' surfaces are painted black to exclude stray light, and overpainted silver to reduce radiation. Further, the upper edges of the box are lined with black velvet to prevent light seepage between the lid and the box.

Holes ($\frac{7}{8}$ in.) are bored at the appropriate height in the walls facing the camera and photographic light and plastic filter holders are fitted. Another aperture of the same design is made in the lid through which the experimental light shines.

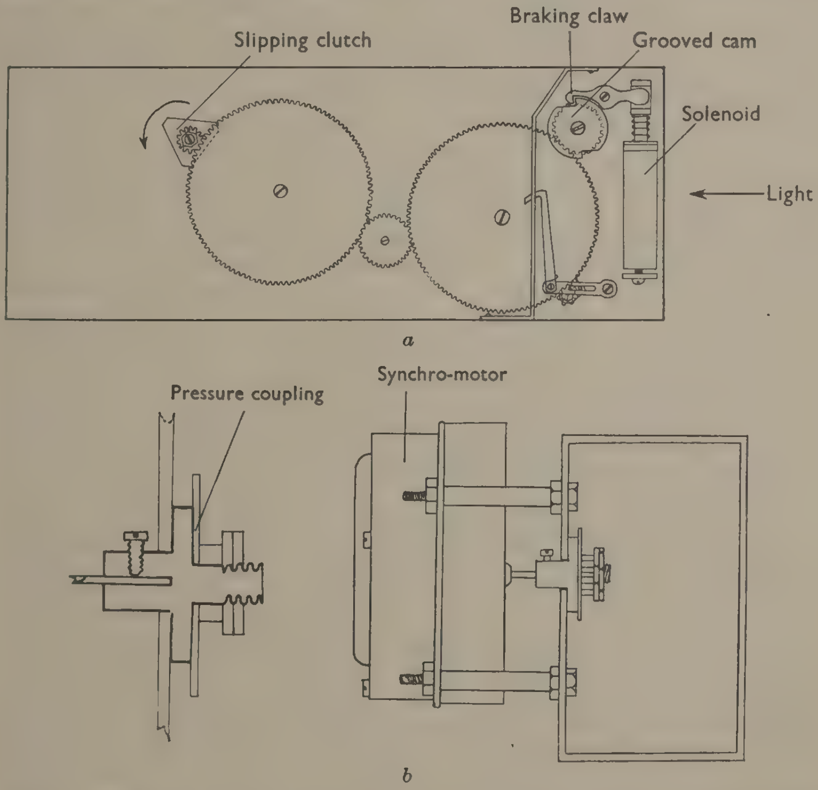
There is no refrigeration device. A 'Sunvic' mercury/toluene thermostat inserted through a hole in the roof controls the temperature through a 6·3 Volt a.c. current heating 18 in. of 35 Ω nichrome wire wound round a



Text-fig. 1. Diagram of the incubator used to photograph *T. elegans* under controlled conditions of light and humidity. *C*, Coverslip window; *cu*, culture of *T. elegans*; *Cy*, cyan filter; *P*, Perspex window; *R*, red filter; *S.M.A.*, synthetic *Mucor* agar; *th*, thermistor; *Ths*, thermostat; *tr*, transistor.

mica gate. The air is kept mixed by means of a spindle, inserted through the box, on each end of which there is fixed a brass disk $3\frac{1}{2}$ in. in diameter and $\frac{1}{32}$ in. thick, cut into 14 sectors and bent to form vanes. The device is rotated by the air current from an independent electric fan which plays over the outside of the apparatus, limiting temperature variation to 0·5° C.

The incubator rests freely on a brass table and can be moved in any horizontal direction. The four table legs are extensible, and vertical movement is controlled by an adjustable screw mounted on the lead base beneath the centre of the table.



Text-fig. 2. Diagram of the G.45 gun camera adapted to take time-lapse photographs. *a*, winding-on mechanism; *b*, slipping clutch mechanism.

The camera. Following a suggestion by Dr B. M. Shaffer, a 16 mm. G.45 aircraft gun camera was adapted to take time-lapse photographs. The entire shutter mechanism, lens and camera motor were removed (Text-fig. 2). Originally one revolution of the one-notched cam wheel advanced the film two frames. The first modification was therefore to make a two-notched brass cam wheel so that the solenoid claw engages and brakes the mechanism after one frame only has been wound on.

The second modification was to substitute a 4 rev./min. Smith's synchro-motor for the original high speed 12 Volt d.c. motor. The synchro-motor is bolted to the back of the camera case and drives directly through the clutch mechanism. The clutch, modified from the original, has a pressure coupling which begins to slip after a certain load is reached. Thus when the braking claw has descended on to the cam wheel and caught in one of the two notches, the entire mechanism stops dead and the pressure coupling slips until the motor itself is switched off a few seconds later.

The process-timer. The process-timer for controlling the camera mechanism and the exposure light consists of a one rev./min. Smith's synchromotor directly rotating three 1½ in. diam. Perspex disks ¼ in. thick. Notches of various lengths are cut in each of the three disks and the plunger of a red-faced micro-switch mounted against each disk is released as the notch passes it, thus causing the camera motor, the photographic light or the braking claw mechanism to be switched on. The sequence of events is then: (1) braking claw solenoid switched on and mechanism freed; (2) camera motor switched on; (3) braking claw solenoid switched off and mechanism braked; (4) camera motor switched off; (5) exposure light switched on; (6) exposure light switched off. The exposure is actually made when the camera motor is off, so that the problem of vibration usually encountered in work of this nature is greatly reduced.

The camera itself is mounted on a strong frame of 1½ in. diam. dural bars bolted on to a lead base. One charge of the magazine lasts about 14 hr. and in practice the device runs continuously during that time.

Optical and photographic. The photographic light consists of an 8 Volt 48 Watt microscope lamp with a condenser, variable both in aperture and focal length, set approximately 1 ft. from the culture. Two ⅛ in. thick sheets of Leitz heat-absorbing glass are placed directly in front of the condenser. One Chance O.R. 1 glass filter with no transmission below 600 $m\mu$ is placed in each of the filter holders on the walls of the incubator. The microscope consists of a brass barrel screwed directly on to the original forward casing of the camera. The objective is a 2 in. Beck and there is a Leitz 'compens-ocular' × 18 eyepiece. The view-finder consists of a prism fixed on to the end of a hollow brass slide mounted on the fore panel of the camera case, just in front of the gate. The image is focused on to the eye with a Leitz 'compens-ocular' × 4 eyepiece with the eye-lens removed.

Ilford Pan F negative ciné film is used as it has a high red sensitivity and a fine grain. In practice the definition of the lens system, which is dictated by the necessity for having as long a focal length as possible, is a little worse than the definition of the film.

Control of illumination. A tungsten lamp (1900 Lux) similar to the photographic light is used as the light source for the illumination of the culture. The lamp is clamped in a fixed position so as to shine through the window in the lid and to be just off-focus at the level of the culture. Two ⅛ in. thick sheets of Leitz heat-absorbing glass are placed in front of the condenser. Originally it was thought that an Ilford 'Cyan' gelatine filter transmitting no light of a wavelength longer than 600 $m\mu$ would be necessary, so that the experimental light would not interfere with the photographic record, but after the first series of experiments this filter turned out to be unnecessary and it was discarded.

The light was checked for constancy by means of the Mullard O.C.P. 71

phototransistor. Although this instrument has many disadvantages (Beament, 1958), its very small size makes it peculiarly suitable for this work. The phototransistor was mounted together with the thermistor, described in the next section, in a probe arranged in such a way that the instruments lie just above and to each side of the fungal colony. Because the phototransistor was found to be a difficult instrument to use, in practice it was used only as a check on the constancy of light intensities. Reliance was placed on the accuracy claimed by Messrs Ilford for their neutral gelatine filters, which were not frequently used, and in the provision of the focusing arrangement which keeps the fungus colony in a constant position in relation to the light. The neutral filters were clipped to the filter holder on the lid of the incubator.

Temperature measurement. The temperature was measured by means of a thermistor with a resistance of about 2000 Ω at 20° C., mounted together with the phototransistor in the instrument probe. The circuit used was modified from Beament (1958). The sensitivity of this instrument in my circuit is about the same as that of a mercury thermometer calibrated in degrees Centigrade. It has the advantage, however, of small size, remote reading and a low capacity for heat. It proved to be a reliable and convenient heat-measuring device.

EXPERIMENTAL RESULTS

Three main types of experiment using the time-lapse techniques have been carried out: (1) the effect of different periods of light of the same intensity; (2) the effect of prolonged illumination at different intensities; (3) the effect of a standard 2 hr. period of illumination at different temperatures.

The reactions of the growing fungus to these treatments are described below. It must be pointed out that in a colony growing on such a small area, such differences as the size of the stab inoculum, the distance of a suitable field from the point of inoculation and the time at which a spike suitable for photography appears make direct comparison from colony to colony a matter for caution.

The fungus responds to illumination in at least three ways: (1) in the number of sporangia produced; (2) in the time a Stage I sporangiophore continues elongation before it produces a fruiting structure; (3) the fungus is positively phototropic and following illumination in both Stage I and Stage IV sporangiophores there is a temporary decrease in growth rate.

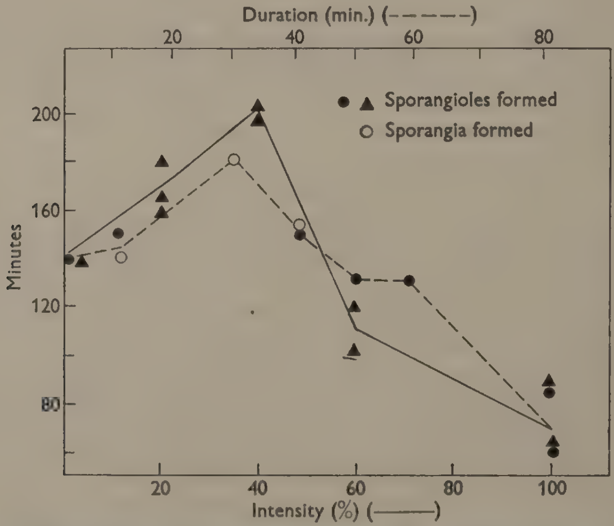
There is little evidence to suggest any inter-relation between these three types of response and they will therefore be considered separately.

Light-induced production of sporangia

This effect has not been studied in detail as the present methods are too laborious to allow a large number of structures to be examined by the statistical techniques that are clearly demanded. Nevertheless, at the relatively high temperature of 22·5° C., at which most of these experiments were carried out, sporangia were only produced in cultures that had been illuminated. The Stage I sporangiophore seems to produce sporangia in response to light only if it is illuminated during the first 50 min. of its upward growth. Thus of the twenty-two sporangia studied at 22·5° C., none was produced by unilluminated cultures, and twenty-one were produced by sporangiophores which had received illumination during their first 50 min. of upward growth. One sporangiophore which did not appear in the photograph until 160 min. after the 10 min. illumination period had finished did, in fact, produce a sporangium.

Influence of light on the duration of Stage I sporangiophore elongation

Banbury (1959) has pointed out that the duration of Stage I growth in *Phycomyces* is shorter in illuminated cultures, and in *T. elegans* the duration of Stage I elongation is also dependent on light. Experiments were designed to show the effect of various quantities of light on the rate and duration of Stage I elongation in *T. elegans*. In these experiments the period of illumination was begun when the sporangiophores to be studied had been growing upwards for not more than 50 min. The results indicate that the duration of Stage I growth is indeed dependent on the illumination the sporangiophores have received, but under these experimental conditions, at least, the relationship between the two is not simple.



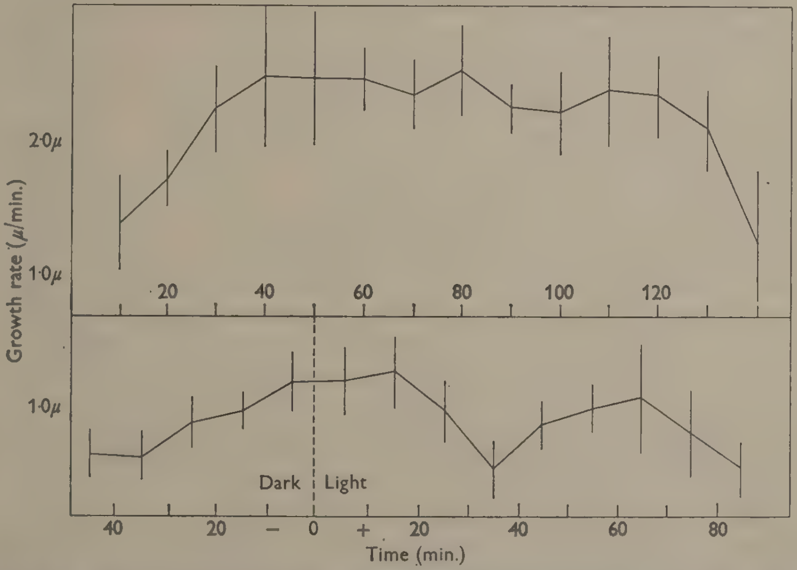
Text-fig. 3. Relationship between the duration of Stage I elongation and illumination of different intensity and time. Sporangiophores growing at 22·5° C.

On the other hand, the results obtained by altering the duration of a high standard intensity and from using prolonged periods of illumination at a fraction of this intensity (Text-fig. 3) show considerable similarity. This suggests that it is the total quantity of light falling on the sensitive sporangiophore that in part determines the duration of Stage I. Nevertheless, the long periods of time involved and the evident complexity of the situation makes it uncertain that such a mechanism should alone be in operation.

The situation just described holds whether the sporangiophore ultimately produces sporangia or sporangioles. The same is true of the slowing of growth rate following illumination, which is described in the next section.

Effect of light on growth rate

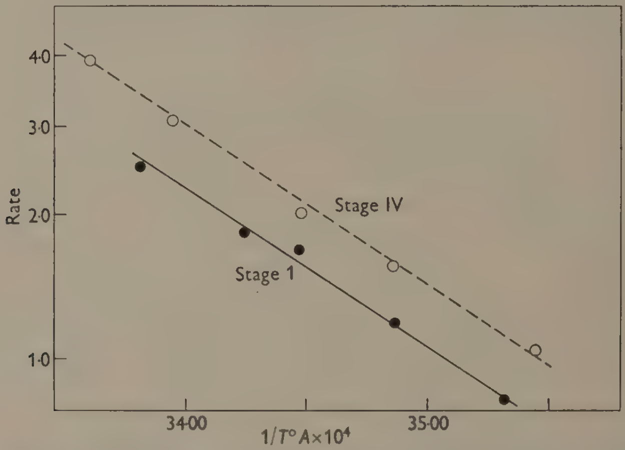
T. elegans shows a temporary decrease in growth rate following illumination in both Stage I and Stage IV elongation. This change of rate has been demonstrated in these experiments by measuring the height of a sporangiophore at 10 min. intervals in the enlarged prints from the 16 mm. negative.



Text-fig. 4. Mean growth rate of Stage I sporangiophores growing at 22·5° C. The vertical lines represent twice the standard error. *a*, Sporangiophores growing in the dark; *b*, sporangiophores which have been illuminated.

This corresponds to the growth rate and is presented as the increment in length in μ/min. The normal dark growth rate of *T. elegans* is only one-fifth to one-tenth that of *Phycomyces* so that although the minimum difference in length that can be detected (about 6 μ) is relatively good, the present method is less sensitive to fractional changes in growth rate than is commonly achieved for *Phycomyces*. This situation is made worse by the fact that the change in growth rate following illumination is a decrease, not an increase as in *Phycomyces*.

Text-fig. 4 shows the mean growth rate curves for a series of Stage I sporangiophores which were illuminated during the first 50 min. of their upward growth. For comparison the mean growth rate curve for sporangiophores grown in the dark is presented. The dark growth-rate measurements were made in the cultures before the light was switched on, and the fact that the culture medium would be relatively fresh at the time when the dark measurements were made might account for the higher mean growth rates for unilluminated Stage I sporangiophores. Nevertheless, it is clear from these experiments that light causes a transient decrease in growth rate which reaches a minimum at about 35 min. after the start of illumination. Lower intensities also produce a decrease in growth rate, and although this is less pronounced, the time for the minimum growth rate to develop is approximately the same within the rather wide limits of experimental error that have to be allowed.



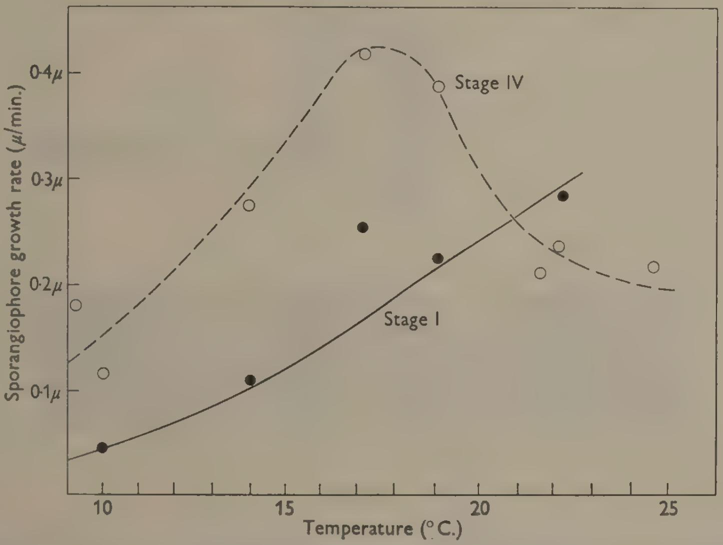
Text-fig. 5. Relationship between the reciprocal of the absolute temperature and the rate of the reactions leading to the light-induced growth rate minimum in Stage I and Stage IV sporangiophore elongation.

Stage IV elongation in *T. elegans* shows essentially the same reactions to light as does Stage I. It differs, however, in that the growth rate minimum consistently occurs sooner after the beginning of illumination than it does in Stage I. This is more fully described in the next section. A further point of difference, which is harder to characterize, is that in Stage IV the growth rate decrease sometimes does not fully recover to its original dark level.

Effect of temperature on the light-induced growth-rate decrease

Although the timing of the light-induced growth-rate minimum is not greatly affected by the light intensity, it is markedly delayed by low temperatures. In these experiments the growth rates of all the sporangiophores in the field of view were averaged and in this way the time of the greatest depression in growth rate was calculated to the nearest 5 min. In every case the depression occurred earlier in Stage IV sporangiophores than it did in Stage I. The reciprocal of the time interval between the beginning of illumination and the time of the maximum depression of growth rate is taken to represent the over-all rate of the reactions involved. The logarithm of this calculated rate is proportional to the reciprocal of the absolute temperature in both Stage I and Stage IV (Text-fig. 5), and although the Stage I depression consistently occurs later the two lines are parallel. The over-all Q 10 for the reactions in both stages is 2·56 as compared with Castle & Honeyman's (1933) figure of 1·7 for the rate of the reaction involved in the lag phase in Stage IV of *Phycomyces*.

The mean growth rates for both Stage I and Stage IV in the various experiments run at different temperatures are shown in Text-fig. 6. Up to about 19° C., Stage IV grows at roughly twice the speed of Stage I; above that temperature there is a suggestion that the optimum conditions for Stage IV have passed. It is clear from these results that the time at which the light-induced growth-rate minimum occurs is not closely related to the growth-rate of the sporangiophore before illumination.

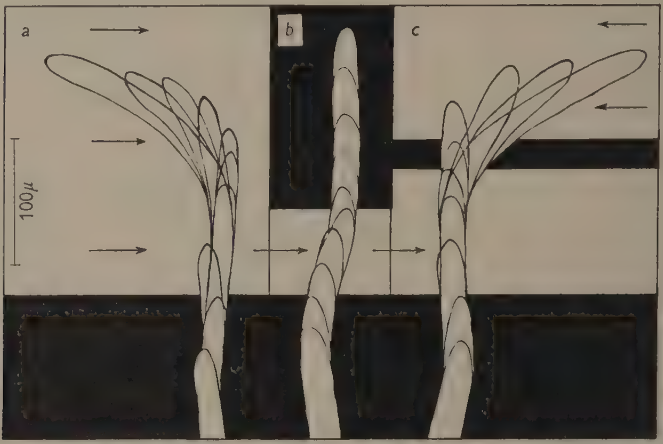


Text-fig. 6. Relationship between temperature and the dark growth rate of Stage I and Stage IV sporangiophore elongation.

Phototropism

These experiments on the phototropism of *T. elegans* were carried out using a Petri dish with a plane glass lid. A cube of agar was cut out and placed on to the main agar surface in the dish. The cube was inoculated and the sporangiophores grew out horizontally from the cube. The Stage I sporangiophores were then drawn at 10 min. intervals, using a camera lucida. The temperature was 23 ± 1° C. There was no specific humidity-controlling mechanism. The light sources were the same as those described for the time-lapse work.

When a light is shone on a sporangiophore from one side, there is no marked phototropic curvature until about 40 min. after illumination (Text-fig. 7*a*), that is after the growth rate has reached its minimum. If, on the other hand, the lateral illumination is stopped after the growth rate minimum is reached, there is no phototropism (Text-fig. 7*b*). The next experiment is to shine light on the sporangiophore from one direction until the growth rate appears to be at a minimum; then after 10 min. in darkness, light from another direction is shone on the sporangiophore. The sporangiophore bends towards the second light (Text-fig. 7*c*). The lag before phototropism towards the second light begins is relatively short and varies between about 5 and 15 min.



Text-fig. 7. Camera lucida drawings of phototropism in Stage I sporangiophores. Outlines are drawn at 10 min. intervals. The arrows show the direction of the light.

These results strongly suggest that the phototropic response is divided into two stages: (1) an un-orientated reaction associated with the slowing of the sporangiophore growth rate, which causes the cell to become sensitive to laterally asymmetric illumination; (2) the phototropic response proper where the cell has become sensitive to laterally asymmetric illumination, and which is associated with an increase in growth rate.

Droplet formation

Castle (1942) has published photographs of droplets on the growing region of Stage I sporangiophores of *Phycomyces*. He states that droplet formation is typically present in Stage I growth, but in no other stage he has observed. He suggests that drops may be of a similar nature to those produced by the subsporangial region of *Pilobus*.

T. elegans produces droplets also, but only during Stage IV. These droplets, which are in some cases more than half the diameter of the sporangium, are regularly produced between one frame and the next, that is, well within the minute. On the subsporangial region, however, the droplets take longer to appear and they tend to be smaller. These droplets act as naturally occurring markers for the rotation of the sporangiophore, which begins in a dextral direction in the same way as *Phycomyces* (Castle, 1942). Reversal of rotation has not been observed in *T. elegans* but this may be due to the fact that droplets are only produced during the early stages of elongation and persist for less than 2 hr.

DISCUSSION

Light and temperature both have pronounced effects on the morphogenesis of the sporangiophore of *T. elegans*. Light has been shown to affect the morphogenesis of the fungus in three ways: (1) it causes a temporary decrease in growth rate and a positive phototropic curvature; (2) it induces the production of sporangia; (3) it affects the duration of Stage I elongation.

Temperature differences affect the morphogenesis of *T. elegans* in many ways but temperature is only considered in its effects which are described below: (1) low temperatures induce the production of sporangia; (2) the optimum temperature for elongation for Stage I lies somewhere above 22·5°C ., whilst the optimum for Stage IV is about 18° C.; (3) the log of the over-all rate of the reactions started by light, and resulting in a temporary slowing of the sporangiophore growth rate, is proportional to the reciprocal of the absolute temperature.

An interesting aspect of this work has come in the study of growth rate changes following illumination and the relationship between these changes and phototropism. At this stage it is not possible to compare the various stages in the light-growth responses in *Phycomyces* and *Thamnidium* because it is impossible to say for certain which, if any, stages in the responses are physiologically homologous in the two organisms. In *Thamnidium*, moreover, the situation is likely to be complicated by the light-sensitive mechanism which is responsible for the preferred production of sporangia in the light.

The presence of a light-induced slowing of growth rate, coupled with a positive phototropism, is of great interest in the study of phototropism. At first sight the occurrence of these two responses in one organism suggests that the lens effect, well established for *Phycomyces*, can not occur in *Thamnidium*. Reversal of phototropism can be produced, however, by growing the fungus under paraffin and this is strong evidence that the lens effect is in operation. Further investigation on this point has shown that the phototropic bending does not begin until the time of minimum growth rate is passed and the sporangiophore growth rate is increasing.

The experiments on the phototropic response itself indicate that, during the time between the beginning of illumination and the slowing of the growth rate to a minimum, there is an un-orientated sensitization of the cell wall to laterally asymmetric illumination. There then follows the phototropic curvature itself, which is sensitive to the lateral asymmetry of the illumination.

The events that occur between the beginning of illumination and the slowing of growth rate to a minimum are obscure. If the over-all rate of the reactions involved in the light-growth response is estimated as the reciprocal of the time lag between the start of illumination and the time of the greatest light-induced growth-rate depression, then the logarithm of this rate is proportional to the reciprocal of the absolute temperature, in the manner empirically demonstrated by Arrhenius (1889) for the effect of temperature on the rate of single chemical reactions (Text-fig. 5).

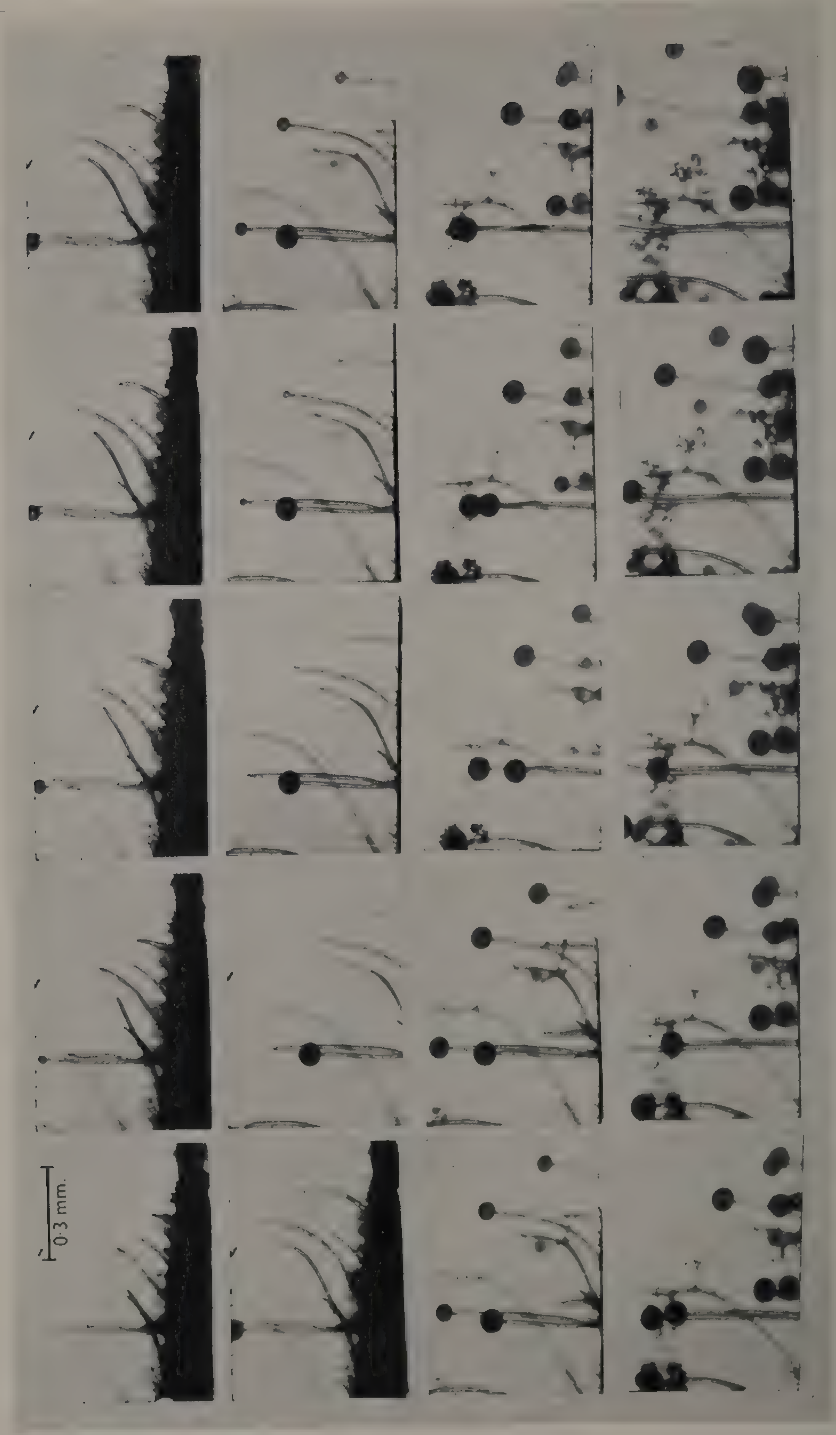
Although the correspondence is interesting, it is not evidence that only one reaction occurs in the time between the beginning of illumination and the growth rate minimum. In the first place, there are many supposedly complex reactions which show a straight-line Arrhenius plot, such as the frequency of flashing in fireflies and the frequency of chirping of crickets (Crozier, 1924). In the second place, the experiments described here were only measured to the nearest 5 min., so that there might be no evidence of a reaction completed within that time.

Production of sporangia. Both light and low temperatures are capable of causing the production of sporangia. No work has been done on the effect of temperature changes at different stages of the fungus's growth, as the aim has always been to keep the temperature constant. Light, however, appears to be effective in causing sporangia to be produced only during the first 50 min. of Stage I elongation at 22·5° C. This might mean either that the light-sensitive mechanism is absent after this time or, perhaps more likely, that the chain of reactions leading to sporangiole formation has gone too far to be reversed by light after about 50 min.

It may be of interest that the sporangia of *T. elegans* react to differences in light, temperature and relative humidity in much the same way as do the conidia of *Choanephora cucurbitarum* (Barnett & Lilly, 1950, 1955). One difference between Barnett & Lilly's strain of *C. cucurbitarum* and my strain of *T. elegans* is that *T. elegans* does not need a dark period after illumination for development.

Duration of Stage I. Although light certainly affects the duration of Stage I elongation, the precise relationship between illumination and the duration of Stage I must remain doubtful. The apparent agreement with the reciprocity law over such a long period of time is likely to be fortuitous. The technique of only beginning illumination when the sporangiophore to be studied has begun to grow might also lead to a confused picture, as the light-induced reactions tending to produce sporangia and a slowing of growth rate might easily interfere with the duration of upward growth in Stage I. On the other hand, the ciné film of the development of illuminated Stage I sporangiophores shows that the slowing of upward growth leading to sporangium or sporangiole production, occurs at nearly the same time throughout the culture (Plate 16).

The precise role played by these light and temperature effects in *Thamnidium elegans* needs a proper physiological and ecological investigation. It would be necessary to find some well defined habitat in which the fungus was endemic before anything more than a guess could be made. It is fairly simple to speculate on the use of, for instance, the Stage I phototropic response; it is more difficult to decide whether there is any ecological significance in the fact that sporangia are produced in the light and sporangioles in the dark. It would, then, be necessary to take into account the fact that a sporangium, initiated at one time under one set of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conditions, would be undergoing Stage IV elongation and would be becoming mature at another time under different conditions.

This investigation shows only some of the ways in which light and temperature affect the morphogenesis of *T. elegans*; it shows little of the physiological mechanisms involved, or of the role of these responses to light and temperature in the adaptation of the organism to its environment.

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EXPLANATION OF PLATE 16

Thamnidium elegans. Photographs, made at 40 min. intervals, of the developing sporangia and sporangioles in a colony growing at 17° C. Illumination is begun at the second picture.

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THIELAVIA RETICULATA SP.NOV.

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(With 1 Text-figure)

Thielavia reticulata sp.nov. isolated from a carnation stem is described. It differs from other species of *Thielavia* in the large reticulate ascospores.

This fungus was isolated on 2 % potato-dextrose agar from stem sections of a 14-month-old carnation plant, cultivar Northland. It was a healthy plant, growing in soil steam-sterilized before planting, and the stem sections were cut from 2 to 4 in. above soil level and surface sterilized in 7 % calcium hypochlorite before plating. The *Thielavia*, which grows very rapidly, was the only fungus to develop from sections of this particular plant.

Since the isolate was first obtained in February 1960, there has been a marked decrease in the number of fertile ascocarps formed on certain artificial media. Thus, in March fertile ascocarps formed in about 10 days on both 2 % potato-dextrose and 2 % plain agar. In April subcultures on plain agar produced no ascocarps, and those on potato-dextrose agar developed more slowly than on a soil-extract agar containing yeast, or on malt and oatmeal agars, where the original vigour was maintained. During May fertile ascocarps were not found in subcultures on potato-dextrose agar, though they continued to be produced on soil-extract and malt agar.

The above observations may reflect the need to add to the medium some growth factor, which the fungus has only limited ability to synthesize or carry over. Similar observations were made by McCormick (1925) and Dade (1938) for *Thielavia basicola* and *T. setosa*, respectively.

Description of cultures

Ascospores do not germinate readily, though a small percentage will do so after about 48 hr. on agar or in water, by the production of a hyphal primordium through the basal germ pore.

Pure cultures were obtained by the isolation of hyphal tips. From these a sparse white to grey mycelium developed which grew both superficially and immersed in the agar. The component hyphae were very variable, being 3·5–12·5μ diam. with cells 25–33μ long. These cells may be constricted at the septa and have, therefore, a doliform appearance. The apparent regular septation of the hyphae is caused by circular thickenings of the wall, for protoplasmic streaming occurs with little hindrance at these thickenings or pseudosepta.

The *ascocarps*, which arise as aggregations of dark mycelium, eventually cover the whole surface of the agar and are superficial or partially immersed. They are dark brown to black with smooth walls, measure 120–220μ, usually 200μ, diam., and have dark brown hyphae radiating from the basal part. In section the wall is 18–20μ thick with two distinct regions: the outer is formed of two to three layers of oval, dark brown, thick-walled cells 9–13 × 4–8·5μ; in surface view these cells are roughly hexagonal and measure 12–20μ across; the inner region is formed of three to four layers of fusoid thin-walled hyaline cells 10–18 × 3–4μ which become somewhat compressed in the mature ascocarp.

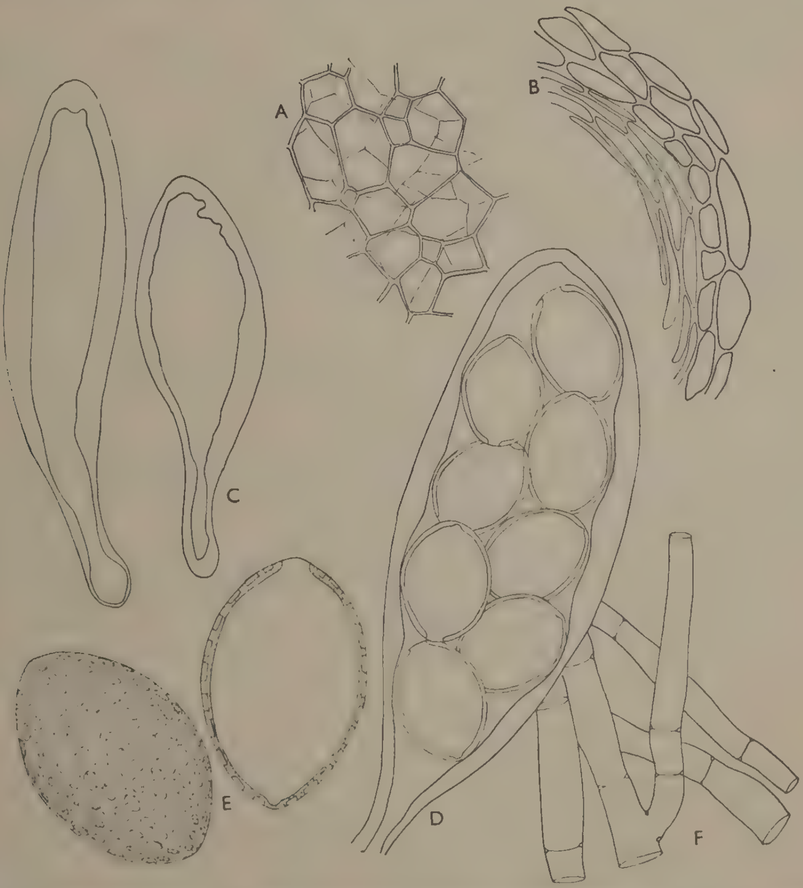

Fig. 1. *Thielavia reticulata.* A, Surface view of part of outer wall of ascocarp; B, T.S. of part of lateral wall of ascocarp; C, young asci; D, ascus with ascospores; E, mature ascospores; F, hyphae. A–D and F = × 750; E = × 1000.

The *asci* form a fascicle arising from the base of the ascocarp; they are clavate with eight monostichous to distichous ascospores. When young the asci are thick-walled and hyaline but the wall becomes thinner as they mature; they measure 130–170 × 25–42 μ.

The *ascospores* are thick-walled, oval or occasionally limoniform, with a germ pore at each end. When mature they are dark brown and develop large pits which give the wall a reticulate appearance; they measure 25–36 × 20–25 μ.

No conidial state has been observed in any of the cultures.

Thielavia reticulata sp.nov.

Mycelium sparsum, album, superficiale et immersum. Ascomata superficialia, nigra, globosa, 120–220 μ in diametro. Paries pseudoparenchymaticus. Asci clavati, cum 8 sporidiis 130–170 × 25–42 μ; primo monostichis dein subdistichis. Ascosporae ovatae vel limoniformes, nigrae, 25–36 × 20–25 μ. Paries crassus et foveatus cum poris germinationi verticibus ambobus.

Habitat in caulibus Dianthi caryophylli cult., Littlehampton, Sussex, legit M. H. Ebben, 4 March 1960. Typus Herb. I.M.I. 80035.

Our thanks are due to Miss S. Daniels for correcting the Latin diagnosis.

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STUDIES ON GRAMINICOLOUS SPECIES OF *HELMINTHOSPORIUM*

I. *H. PHLEI* (GRAHAM) COMB.NOV.

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(With Plates 17 and 18 and 2 Text-figures)

Since Drechsler (1923) published the results of his studies on some graminicolous species of *Helminthosporium*, no one has studied in detail a large number of species on grasses at the same time. In some species there is a great variation in dimensions and other characters under different environmental conditions. Especially variable are those species which produce their conidia in chains. In this paper one of these variable species is described in detail. Later papers will deal with several other species.

Helminthosporium phlei (Graham) comb.nov.

syn. *H. dictyoides* Drechsler var. *phlei* Graham, 1955, *Phytopathology*, **45**, 227.

Graham (1955) described *H. dictyoides* var. *phlei* as the cause of wither tip, chlorotic blotch and leaf streak on timothy (*Phleum pratense* L.) in N. America. The report of the National Institute of Agricultural Botany, Cambridge, for 1955 recorded *Helminthosporium* sp. producing striping and blotching of the foliage of timothy grass. Most of the 200 timothy seed samples examined at that Institute contained some infection, a few up to 30 %, and some of the infected seeds failed to germinate. As part of a major study of *Helminthosporium* diseases of grasses, an investigation of these two fungi was undertaken.

MATERIAL EXAMINED

(1) A sample of infected timothy seed from Cambridge.

(2) Diseased timothy leaves collected by J. H. Graham in August 1957 at State College, Pennsylvania.

(3) A slide of *H. dictyoides* var. *phlei* prepared from a piece of diseased timothy leaf collected by Dr Mary Noble at Tillicoultry, Scotland, 6 August 1956, placed in a moist chamber.

(4) *H. dictyoides* var. *phlei* from timothy seed, Denmark, Andersen (598–HA–14/6/55).

(5) *Helminthosporium* sp. ? *catenarium* from timothy seed, 25 March 1955 (source unknown).

(6) *Helminthosporium* sp. from American timothy, 28 March 1949, S.T.S. 9431 (source unknown).

(7) *Helminthosporium* sp. from timothy seed, Finland, prz 329, 1955.

(Nos. 4–7 were slides from Dr Andersen (Neergaard, 1956), Denmark.)

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Some of the infected seeds were sown in the field to study the fungus in nature and some were germinated on moist filter-paper in Petri dishes to determine the percentage infection and to study the fungus on seeds and seedlings. Isolates were used for inoculations. The material from Pennsylvania was used to study the fungus on its natural host, in culture and for inoculations. Slides were prepared from single infected seeds or seedlings mounted whole in lactophenol or cotton blue in lactophenol; others contained pieces of an infected leaf bearing fructifications produced under field conditions or in a moist chamber.

SYMPTOMS

On the leaves irregular, light brown, necrotic streaks or blotches with a conspicuous chlorotic border are produced. These necrotic areas, usually 1–5 mm. wide, are often marginal and may extend the length of the leaf. In a severe attack coalescence of lesions causes browning of most of the blade. Sometimes necrotic and chlorotic blotches appear on the sheath and the whole blade withers and dies. In mature leaves the entire distal part of the leaf, together with the margins of the lower part, may be necrotic, the only part of the leaf remaining green being an irregular band along the midrib in the lower part. In an infected flowering plant the older leaves are usually most infected, while the upper leaves may be only partially necrotic. Later, the disease may extend to the inflorescence.

Apart from the large necrotic areas, there is also another type of lesion on timothy grass. During the summer small elongated purple spots, 1–$3 \times \frac{1}{3}$–1 mm., appear on parts of the blade. Some of the larger spots have a discoloured point at the centre, and are typical eye spots. Later, the whole of each eye spot gradually loses its purple colour and becomes light brown. At the same time the area round the eye spot becomes chlorotic and extends to form a larger necrotic blotch. As a result of increasing density, coalescence and maturity of the lesions these plants eventually show the general necrotic symptoms of the disease. It is assumed that these eye spots are the first reaction of the plant, each being a mild infection by a single spore at a time when conditions are unfavourable for rapid development of the fungus.

THE FUNGUS ON THE PLANT

Definition of terms

The term *conidiophore* is applied to a sporophore emerging from the tissue of the host or from the side or distal end of a hypha.

The term *secondary conidiophore* is applied to a sporophore emerging from a cell of a mature conidium (primary spore) or, at right angles and usually with a marked base, from a mature conidiophore.

The *first part* of a conidiophore is the length from the base up to the first scar (place of attachment of the first conidium).

The *second part* of a conidiophore is the length from the first scar up to the apex of the conidiophore.

The terms *first conidium*, *second conidium*, *third conidium* etc. are used for first, second and third spores of a chain (Text-fig. 1 B, *e*, *f*, *g*) counting from the base, while the term *secondary conidium* is generally used for a spore produced on a secondary conidiophore emerging from any cell of a primary spore (Text-fig. 1 *h*).

The following description of *Helminthosporium phlei*, based on fructifications in a moist chamber, is compiled from the examination of a number of slides containing more than 100,000 spores; the characters of about 25,000 spores are given in Tables 1–3.

Internal mycelium composed of hyaline septate hyphae, usually 1·5–7 μ thick; *external mycelium* composed of light to dark brown or olivaceous hyphae up to 9 μ thick, usually not thicker than 7 μ, either without any constriction at the septa or with some constriction when small or large pyriform swellings may occasionally be formed.

Conidiophores yellowish, light olivaceous, light olivaceous brown or light dirty brown; they emerge from plant tissue developing from the internal mycelium or sometimes laterally from the external hyphae produced on diseased parts in a moist chamber. They are usually simple, single or sometimes in pairs or rarely in fascicles of up to four. Two opposite secondary conidiophores may emerge from the tip of the first part of the conidiophore just beneath the first scar. Secondary conidiophores often emerge from the cells of a primary conidium, especially from the basal and apical cells, from which two opposite conidiophores may emerge. Occasionally a secondary conidiophore emerges from the hilum. Conidiophores are generally cylindrical, but occasionally they may be an abnormal fusiform shape at the upper part below the first scar (Text-fig. 1 *d*). In the field conidiophores are usually shorter and wider than those produced in a moist chamber. The distance between two scars on a conidiophore varies from 3 to 296 μ, usually up to 150 μ, and the distance between two septa is 10–35 μ, usually 15–25 μ. Other dimensions are given in Table 1.

Conidia. H. phlei produces spores in chains and so there are usually two types of conidia on a slide.

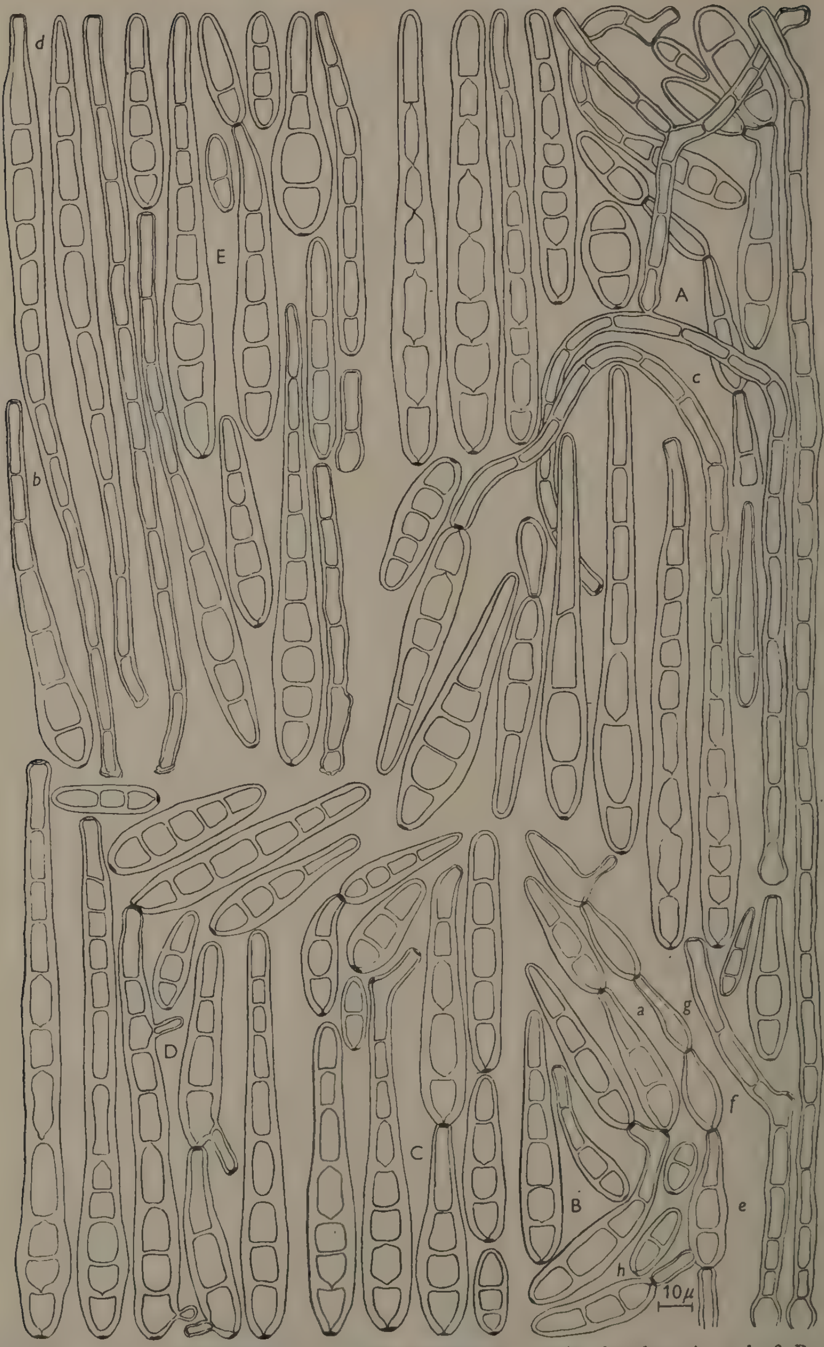
Single conidia, which have either arisen singly from the tip of a conidiophore or constitute the terminal spore in a chain. They have no apical scar.

Scar conidia, each with an apical scar; spores of this type are all those in a chain except the terminal one. They may be divided into three groups:

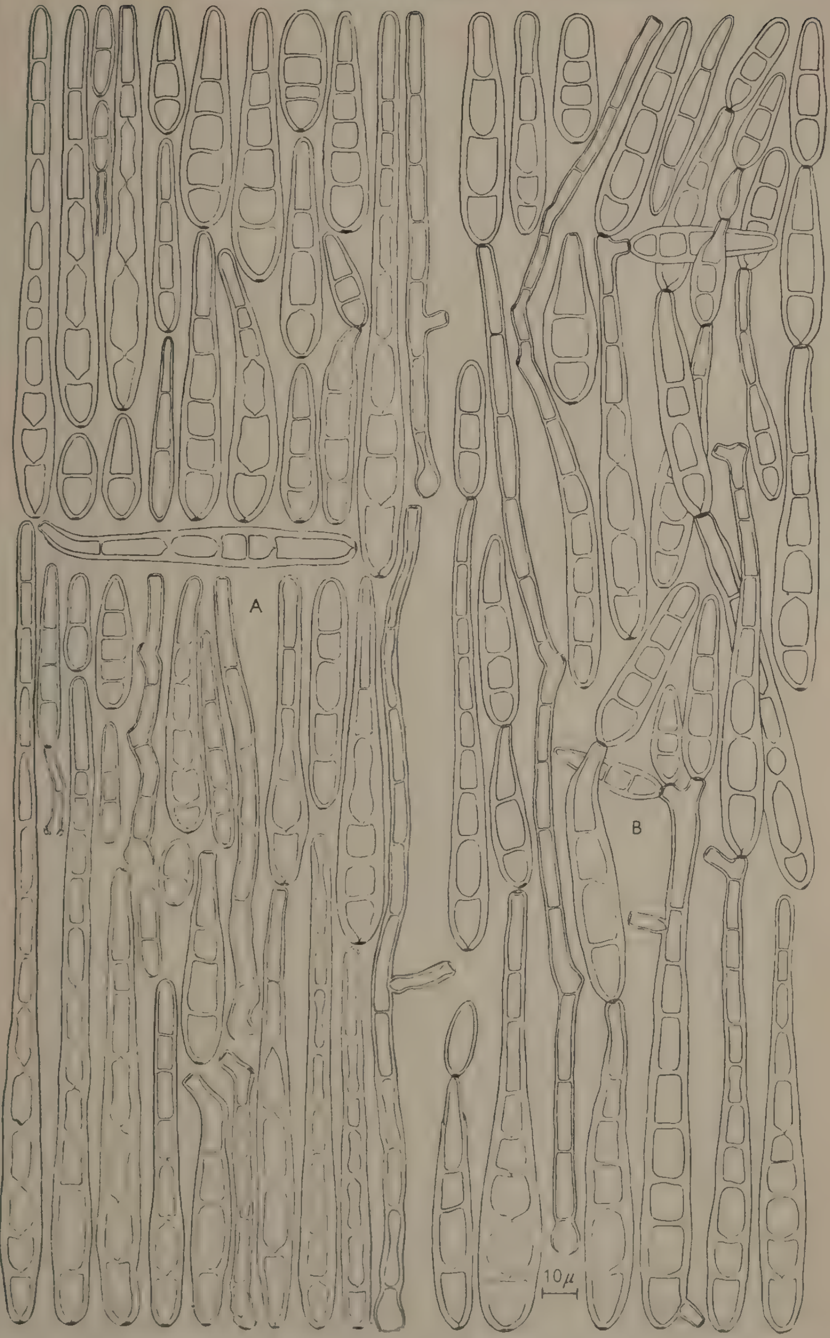
(*a*) Scar conidia without a beak, with usually one apical scar, the apical cell up to the scar being a cone formed as a prolongation of the lower part of the spore. If there is more than one apical scar the length of the spore is regarded as being up to the first scar (Text-fig. 1 *a*).

(*b*) Scar conidia with a short beak; in these the apical cell, if partially or even entirely cylindrical, seems to be a prolongation of the lower conical part of the spore. If there is more than one scar, the length of the spore is taken up to the first scar. Short beaks are not usually longer than 25 μ but may occasionally be up to 41 μ, which is the maximum length of an apical cell in single spores (Text-fig. 1 *b*).

(*c*) Scar conidia with a conidiophore-beak. Sometimes the first spore in a chain, and occasionally the second spore in a chain of more than two spores, develops a long septate conidiophore-like beak, usually with one,



Text-fig. 1. A–D. Fructifications of *H. phlei* produced in a moist chamber: A, on leaf; B, on a diseased seed; C, D, on two seedlings. E. Conidiophores and conidia produced in a moist chamber on leaf material collected at Tillicoultry.



Text-fig. 2. A. Fructifications of *H. phlei* on diseased leaves kept for a few days in a moist chamber in Pennsylvania (Graham). B. The same, but also 7 days in a moist chamber in England.

Table 1. *Conidiophores of* Helminthosporium phlei, *dimensions (in μ) and septation*

		First part of conidiophore (base up to 1st scar)						Second part		Maximum of total		
		Range			Usual dimensions							
		Length	Width	Septa	Length	Width	Septa	Length	Septa	Length	Septa	Scars
1	Seeds (11 slides)	15(77)–183(354)	4(5·7)–7(9)	0(3)–6(19)	55(80)–125(231)	5(6·5)–6·5(7)	1(3)–4(9)	3(19)–48(305)	0–2(14)	193(460)	8(21)	2(5)
2	Seedlings (17 slides)	18(96)–257(612)	4·5(6)–7(9·5)	0(3)–12(31)	80(120)–175(377)	5·5(7)–6·7(8·3)	2(4)–6(22)	6(48)–32(338)	0(1)–1(14)	280(612)	12(31)	1(5)
5	Leaves (5 slides)	28(45)–222(432)	4·5(5)–8(10·3)	0(1)–9(22)	55(80)–160(320)	6(6·5)–7(8)	2(3)–7(13)	4(67)–41(315)	0(2)–0(13)	240(450)	9(22)	2(4)
6	Leaves from field (3 slides)	28(54)–161(466)	5·5(6·5)–8·5(9·5)	1(2)–10(19)	75(80)–125(175)	7·5–8·5	2(3)–6(8)	9(15)–80(100)	0–4(5)	177(466)	10(19)	4(5)
7	Leaf, Tillicoultry (1 slide)	22–290	5·7–11	0–14	50–180	6·5–9	2–8	58–150	1–6	290	14	2
8	Leaves in Pennsylvania (4 slides)	16(48)–177(264)	4(5)–8(8·5)	0(1)–7(12)	40(65)–130(185)	5·7(7)–7(8)	1(3)–5(7)	3(19)–24(77)	0(1)–0(3)	177(264)	7(10)	2(4)
0	Leaf from Pennsylvania in England (1 slide)	32–273	4·5–8·5	1–12	70–180	6·5–7·5	2–8	6–215	0–9	364	17	4
11	7-day-old culture on Langeron GPA (1 slide)	22–232	5–8·5	0–11	75–160	6·5–7·5	3–6	6–200	0–6	383	15	3
12	*Festuca rubra* leaf (1 slide)	99–257	5·7–8·3	2–9	110–240	6·5–7·5	3–8	9–112	0–3	257	9	3

In Tables 1–3 items 1, 2, 5, 7, 8, 10, 12 all in a moist chamber.

Table 2. *Conidia of* Helminthosporium phlei, *dimensions (in μ) and septation*

		No. of conidia on slides	Limit dimensions			Usual dimensions (90%)			Average		
									Length		
			Length	Width	Septa	Length	Width	Septa	Minus beak	Plus beak	Width
1	Seeds (11 slides)	1483	13(28)–74(125)*	6·5(10·3)–14(20·5)	0(1)–4(8)	24(33)–53(88)	9·5(11)–12·8(16·1)	1(2)–3(6)	39·15–59·70	39·15–59·97	11·51–13·22
2	Seedlings (17 slides)	8543	13(32)–83(241)	6·5(9·5)–14·8(20)	0(1)–6(14)	16(42)–76(124)	7·7(12·3)–13(15·8)	1(2)–5(8)	43·05–77·32	47·43–77·53	10·79–13·89
3	One of the slides from 2	885	19–167	8·3–17·4	0–11	40–124	12·3–15·8	2–8	77·32	77·53	13·89
4	Conidia from tip of the above seedling	100	38–167	10·3–17·7	2–11	71–152	12·3–15·8	4–10	112·76	112·76	14·12
5	Leaves (5 slides)	7269	13(22)–138(244)	6·5(8·3)–17·3(20·5)	0(1)–7(13)	34(51)–98(132)	10·4(11·9)–14·5(15·1)	1(2)–5(7)	64·73–79·20	66·40–79·20	12·73–13·86
6	Leaves, field conditions (3 slides)	1016	16(22)–106(109)	8·3(9·5)–16·7(18·6)	0–7(8)	25(36)–79(91)	10·6(11·2)–14·5(15·7)	1–5(6)	52·67–62·10	52·67–62·10	12·84–13·40
7	Leaf, Tillicoultry (1 slide)	336	22–157	7·7–18	1–9	42–114	11–15·4	2–7	73·17	74·70	13·29
8	Leaves in Pennsylvania (4 slides)	1855	13(22)–167(235)	6·5(7·7)–15·5(17·3)	0(1)–9(13)	31(58)–119(145)	8·8(11·4)–14(14·6)	2(3)–7(8)	73·86–94·32	73·93–94·32	12·02–12·68
9	Long single conidia from 8	92	145–235	10·3–15·5	7–13	—	10·7–13·9	7–12	—	—	12·55
10	Leaf of 8, 7 days in moist chamber in England (1 slide)	3070	13–173	6·5–20	0–9	34–123	11·1–14·8	2–7	—	72·18	13·01
11	7-day-old culture on Langeron GPA (1 slide)	500	19–112	8·3–20·5	0–7	26–75	10·3–14·5	1–4	—	50·47	12·52
12	*Festuca rubra* leaf artificially inoculated (1 slide)	120	25–132	8·3–14·8	0–6	35–103	9–13·5	1–4	65·01	65·01	11·59

* 13(28)–74(125) indicates that in a slide the minimum length of conidia may be 13–28μ and the maximum length 74–125μ.

Table 3. *Single conidia, scar conidia and conidiophore-beaks of* Helminthosporium phlei, *limit dimensions (in μ) and septation*

		Single conidia			Scar conidia				Conidiophore-beaks			
		Limit dimensions Range				Limit dimensions Range						
		Length	Width	Septa	%	Length	Width	Septa	%	Length	Septa	Scars
1	Seeds (11 slides)	13(29)–58(90)	6·5(10)–14(17·4)	0(1)–3(6)	5·9–38·2	16(99)–74(126)	9(12.2)–12·8(20·5)	0(2)–4(8)	0–9	22(58)–32(96)	1(2)–1(5)	1–3
2	Seedlings (17 slides)	13(32)–77(116)	6·5(9·5)–14·8(17·4)	0(1)–4(8)	1·2–39·6	18(80)–80(242)	8·3(12)–12·8(17·3)	0(5)–5(15)	0–15·7	16(35)–48(183)	1–3(11)	1–3
3	One of the slides from 2	22–116	8·3–17·4	0–7	39·6	19–167	11·5–17·3	1–11	0·6	35–58	1–3	2
4	100 conidia from tip of the above seedling	38–116	10·3–14·8	2–6	61·0	83–167	12·3–16·3	4–11	0	—	—	—
5	Leaves (5 slides)	13(22)–129(141)	6·5(8·3)–17·4(20·5)	0(1)–7(9)	7–12	22(71)–119(245)	9(11·5)–16(16·7)	0(3)–7(13)	0–5·7	16(35)–58(138)	0(1)–3(6)	1–3
6	Leaves, field conditions (3 slides)	16(22)–96(109)	8·3(9·6)–16·7(18·7)	0–7(8)	0·4–1·7	64(86)–90(106)	11·5(14·1)–13(14·8)	3(4)–4(7)	0	—	—	—
7	Leaf, Tillicoultry (1 slide)	22–132	7·5–17·4	1–9	15·5	48–158	8·4–18	3–9	4·0	35–90	1–3	1
8	Leaves in Pennsylvania (4 slides)	13(22)–167(235)	6·4(7·7)–16(17·4)	0(1)–10(13)	1·4–5·3	42(87)–119(145)	10·9(12·2)–13·5(14·8)	2(4)–6(9)	0–0·3	19	1	1
9	Long single conidia from 8	145–235	10·3–15·4	7–13	—	—	—	—	—	—	—	—
10	Above material, again 7 days in moist chamber in England (1 slide)	13–135	6·4–19·3	0–9	38·5	15–174	6·5–19·3	0–9	14·0	20–103	1–4	4
11	7-day-old culture on Langeron GPA (1 slide)	13–80	8·3–20·5	0–6	17·0	28–112	8–15·5	0–7	0·6	25–48	1–3	2
12	*Festuca rubra* leaf artificially inoculated (1 slide)	25–90	8·3–14	0–4	34·8	28–132	8·3–14·8	0–6	0	—	—	—

sometimes two, seldom three or four, scars. Such a conidiophore-beak is a prolongation of the body of the spore. It starts to grow when the spore itself is very young and completes its growth simultaneously with the body of the spore. In a conidiophore-beak spore there is not usually a marked line between the conidiophore-beak and the body of the spore. This character distinguishes it from a spore which, after becoming mature, has produced a secondary conidiophore from the apex. A conidiophore-beak up to the first scar is also considered to be a part of the spore (Text-fig. 1*c*).

Single conidia are typically straight and conical, usually widest at the first septum or at the second cell and tapering uniformly or with some slight irregularities towards the apex. The basal cell is usually hemiellipsoidal, narrower than or as wide as the next above (second) and rarely (in a few round-based conidia) slightly wider. It is a rare exception that the third cell is the widest. The apical cell is usually conical with an almost rounded apex, but sometimes it may be cylindrical or slightly widened near the tip. Some short 0–3 septate conidia may be short or long ovoid or ellipsoidal and a few of the narrow conidia with 2–5 septa may be cylindrical or subcylindrical. There is usually no constriction at the septa. There is no difference as regards general shape between single conidia and conidia with an apical scar, but in the latter the apical cell, or the upper part of the spore in conidiophore-beak spores, is usually bent under the weight of the successive conidia in the chain. Moreover, conidiophore-beaks are usually slender and flexible.

The wall of the conidium is usually uniformly thick (about 3–4μ) from the base up to the apical cell where it becomes gradually thinner towards the apex. The wall at the base completely envelops the hilum.

The conidia are hyaline at first, then light olivaceous yellowish. They are almost the same colour as conidiophores but usually somewhat paler.

The number of septa and the length vary considerably with the external conditions and the part of the host on which they are produced. On seeds spores are usually shorter than on seedlings and leaves; those formed in the field are shorter than in a moist chamber (see Tables 2 and 3). The distance between two septa ranges from 6 to 28μ and the apical cell may be up to 41μ long and 6·5–12μ wide, usually 8–9μ. Width is not so variable as length (Tables 2 and 3).

Second spores in a chain range from 13–93 × 6·5–20μ with 0–6 septa. If it is one with a conidiophore-beak it may be up to 154μ long with 10 septa and with up to three scars. A short first conidium with no septum may bear a longer second spore with several septa, but usually the second spores are shorter than the first ones. Though second spores may be as wide or even wider than the first spores they are usually narrower (915 second spores measured). Third spores in a chain measure 13–77 × 6·5–14μ, with 0–6 (but usually not more than 3) septa. Very occasionally one may have a conidiophore-beak with one or two scars (dimensions obtained from 91 third spores). Fourth spores in a chain are rare. They measure 25–74 × 9–11·5μ, with 0–3 septa (dimensions from 12 fourth spores). Only one chain with five spores was seen. The fifth spore was 22 × 9·5μ with no septum (Pl. 17, B).

In the field only a small percentage (up to 1·7) of spores may be in chains, while in a moist chamber there may be up to 40 % (Table 3, no. 3).

On germination in water, spores produce one, sometimes two, germ tubes from any of their segments, but germ tubes are produced most frequently from the apical cells.

THE FUNGUS IN CULTURE

Isolates were obtained from diseased leaves and seeds of *Phleum pratense* collected at Silwood Park and from leaves sent by Graham from State College of Pennsylvania:

Media

Wheat flour agar (WFA, 40–20).

Langeron glucose peptone agar (GPA, 20–10–20).

Potato-dextrose agar (PDA, 200–15–15).

Malt-extract agar (MA, 30–20).

Glucose peptone potassium phosphate (KH_2PO_4) magnesium sulphate agar (GPPMA, 10–2–1–0·5–15).

Asparagine maltose magnesium sulphate potassium acid phosphate agar (Coon's synthetic agar = Coon's s., 0·25–3·5–0·5–1·25–12).

L-Proline, KH_2PO_4, K_2HPO_4, KCl, $MgSO_4.7H_2O$, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, sucrose agar (SPA, 2·7–1–1·3–0·5–0·5–0·01–0·002–0·0016–6–20).

In the proportions given, all media were made up in 1000 ml. distilled water.

Macroscopic characters

Two different types of growth were observed:

Normal growth. In plates inoculated in the centre a white moderately woolly growth begins to develop. On the second or third day an olive colour appears starting from the point of inoculation. A growing colony always has a white edge about 1 cm. wide. The edge is regular but the growing hyphae advance sparsely without forming a sharp line. The aerial growth, which at first is loose and woolly, becomes somewhat dense and felty with the development of the olive colour. The whole surface of the plate is regularly covered, so that a culture about 2 weeks old at room temperature (*ca.* 20° C.) has a flat, somewhat smooth and felty surface. At this time it is homogeneously olive or dark olive without any concentric circles. There are usually no coremia or tufts of mycelial growth on the surface of the colony, except in some very old cultures where a few irregularly scattered white coremia or some sparse or agglomerated dwarf felty knowles may develop.

In old cultures immature *Pyrenophora* perithecia developed on all agar media used.

Colony diameters in mm. after 7 days at 20° C. on the different media were:

WFA	GPA	PDA	MA	GPPMA	Coon's s.
75	70	65	60	55	50

Abnormal growth. In most subcultures of all isolates the fungus after producing some normal olive growth with a whitish margin sooner or later stopped growing. Gradually the filaments at the edge of the colony became scanty and dark brown. Such a brown colony was composed of thick brown hyphae with many chlamydospore-like swellings (Pl. 18, L). Inocula from such a mycelium, especially when taken near the edge of the colony, did not grow. If inoculum was taken earlier when there was still growth in the plate, there was some small growth in the newly inoculated plate which very soon ceased.

A large circle of dark olive mycelium usually remained in the centre of a brown colony. Inoculum taken from this region produced either a normal colony which occupied the whole plate or a brown colony which ceased growing. In colonies where both types of mycelium were present the brown mycelium remained underneath, closely attached to the surface of the medium or immersed in it, and the olive mycelium with thinner hyphae occupied a smaller circle on the surface. Because of the presence of both types of mycelium such a colony usually showed concentric circles with an outer dark brown circle where there was no further growth. The inner circles were usually shades of dark or light olive and even sometimes shades of brown or copper colour.

In brown colonies where growth had ceased at the border the olive mycelium from the centre sometimes overran the brown edge and occupied the empty areas around the colony. This over-running mycelium either kept its olive colour or turned in some regions to a brown mycelium, giving a mosaic appearance to the growth around the first brown colony. Inoculum taken from the overrunning mycelium produced either a normal or a brown colony.

The timothy fungus does not produce any pigment on SPA but a grey felty aerial growth and some thin strands in the medium near the surface. On this medium growth is very similar to the growth of the stripe fungus from American tall fescue.

Microscopic characters

Yellowish brown conidiophores may arise at right angles from the sides of hyphae or the distal ends of hyphae may become thicker and darker to form conidiophores; the first part measures 22–515 × 5–9μ, with 0–22 septa, usually 70–160 × 6·5–7·5μ, with 3–6 septa; the second part is up to 300μ long with 13 septa and 9 scars, and the total length may be up to 692μ with 28 septa.

Conidia are of the same shape, colour and appearance as on natural substrata. Single conidia are from 16 to 312μ long, with 0–15 septa, but usually in cultures single conidia longer than 115μ with more than 7 septa are not easily found. These long single conidia consist of the usual conical body terminated by a somewhat flexuous cylindrical upper part which may be up to 215μ long with 10 septa. There are occasionally some club-shaped spores which are narrowed in the middle and widened at both ends resembling two spores in a chain, but with a complete septum between them instead of a hilum. Scar conidia are 22–151μ long with 0–9 septa, with or without a short beak or with a conidiophore-beak up to

96μ long to the first scar, having 5 septa and one to three successive scars. The width of spores varies from 7·5 to 20·3μ. Spores wider than 15μ are occasionally seen. Second spores in chains are 16–61 × 7·5–15μ, with 0–4 septa, and third spores are 7·5–40μ long, with 0–2 septa and up to 13μ wide. No. 11 in Tables 1–3 shows almost typical microscopical characters of the fungus in culture.

In some old cultures patches of a secondary top growth appear. Macroscopically this looks like a *Cladosporium* infection. The patches are about ½ mm. high with a flat compact surface and a homogeneous olive colour. Microscopically they are formed of densely compacted short septate wavy filaments, similar to conidiophores of *Polythrincium*; they may be simple or branched several times. These filaments sometimes have irregular chlamydospore-like swellings and usually somewhat screw-like, tapering or round ends.

Inoculations

Potted greenhouse plants were sprayed when about 10–20 cm. tall with a suspension of mycelium and spores from ground up cultures filtered through fine muslin. The test was done four times between 16 April and 10 July 1958. Three isolates were used: the Silwood blotch and eye spot isolates and one from Pennsylvania material.

Results

All three isolates were similar in culture and gave similar results. After 1 or 2 weeks leaves with lesions or some wither tip were collected and placed in a moist chamber. The appearance of fructifications after a few days was regarded as a positive result.

On timothy there was always heavy infection with characteristic symptoms, withered and chlorotic blotches, and sometimes a few eye spots. The fungus is more specific to timothy than are *H. dictyoides* or *H. siccans* Drechs. to their respective hosts.

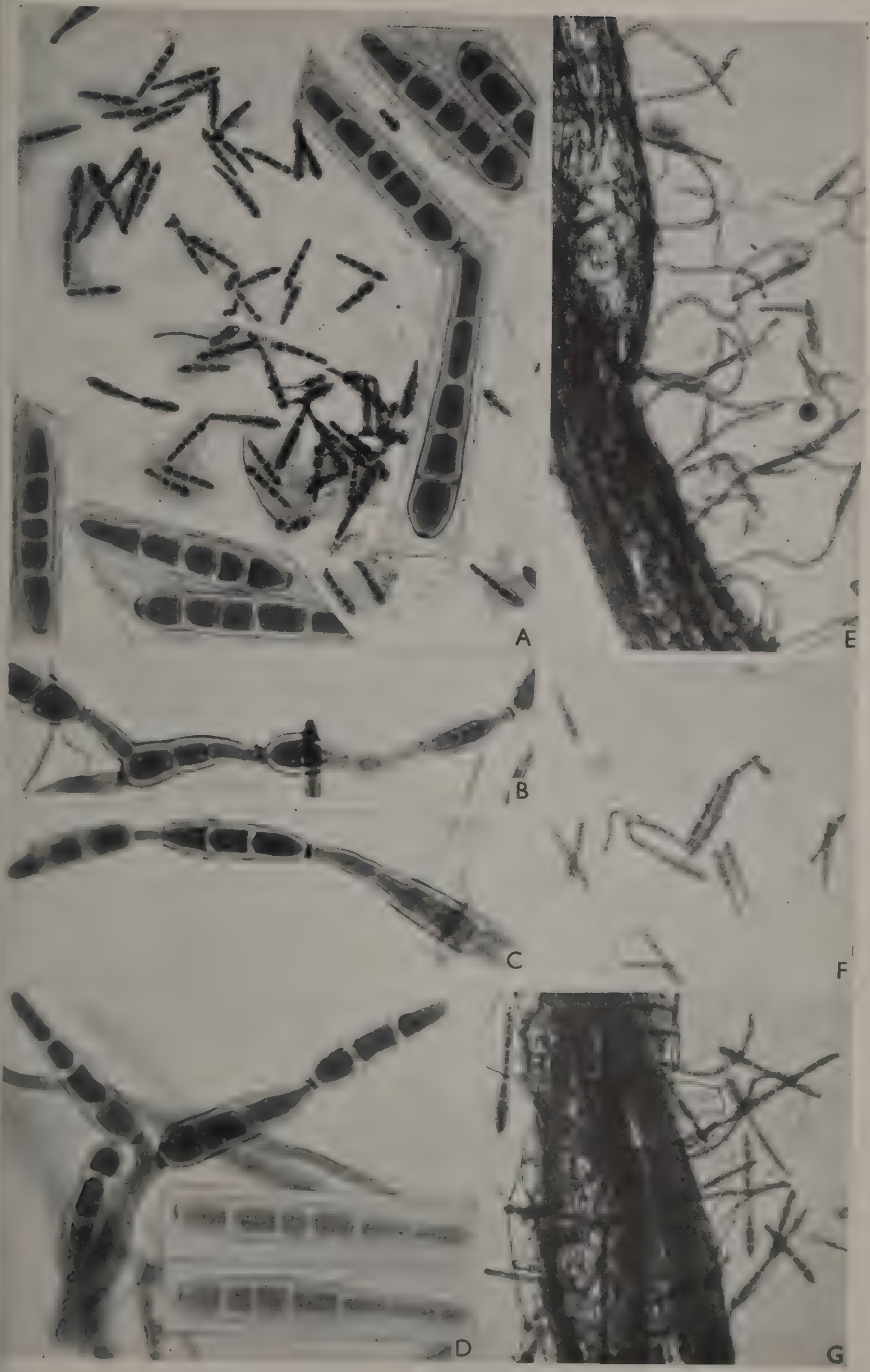
On fescue (*Festuca rubra*) a few chlorotic and withered blotches appeared which in a moist chamber produced numerous fructifications (Tables 1–3, no. 12). This host was only slightly susceptible.

On American tall fescue (*F. elatior* subsp. *arundinacea*), Italian and perennial rye grass (*Lolium italicum* and *L. perenne*), no definite symptoms were produced, but naturally withered leaves from the inoculated plants produced a few fructifications in a moist chamber.

No symptoms developed on meadow fescue (*F. pratensis*), wheat, barley or oats and no fructifications were produced on inoculated leaves placed in a moist chamber.

Discussion

The *Helminthosporium* on timothy at Silwood is identical with *H. dictyoides* var. *phlei* and with the fungi from Scotland, Denmark, Finland, etc., which produce catenate spores on timothy. Though there is some similarity to *H. catenarium* Drechsl., *H. dictyoides*, and to an undescribed *Helminthosporium* species in having tapering and catenate spores, there are many differences in pathogenicity, cultural characters and morphology. Spores



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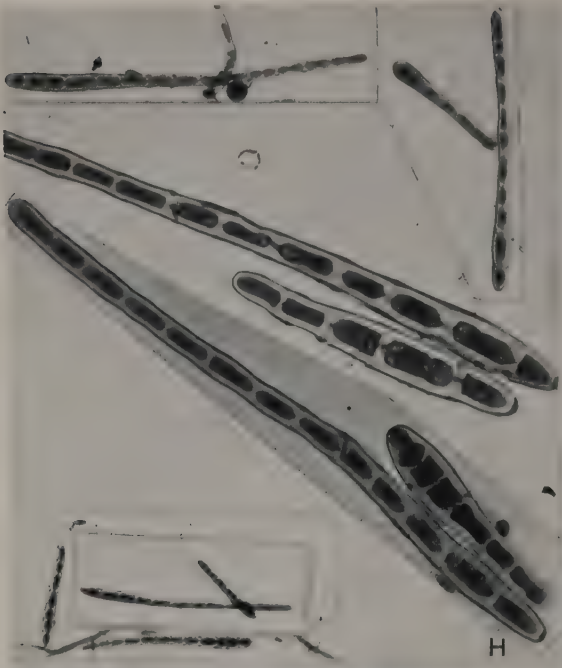
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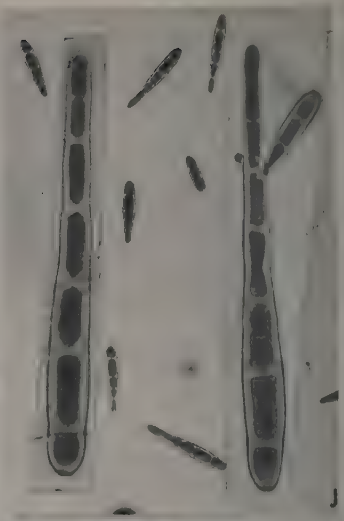
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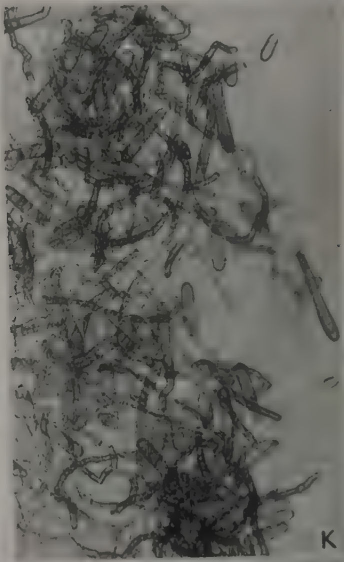
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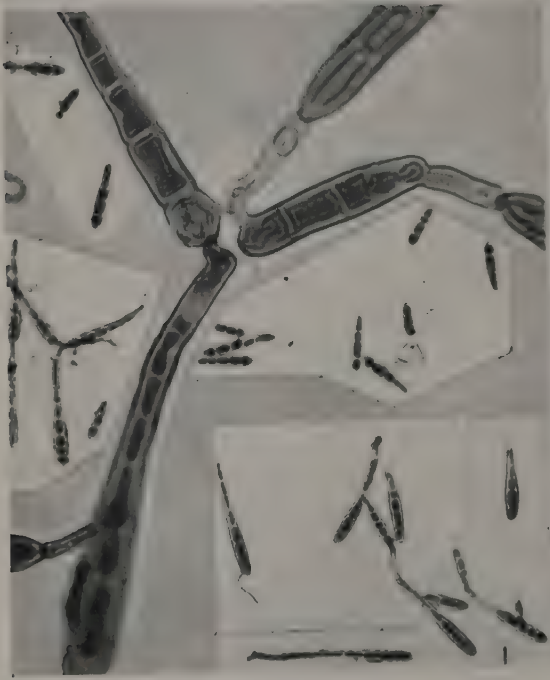
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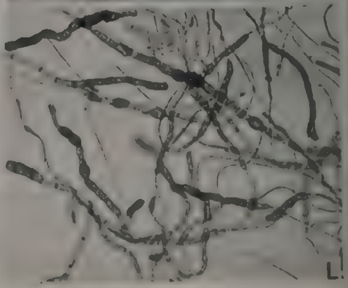
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of *H. phlei* are always narrower than those of the other four species, the mean spore width of the former being always less than 14μ (10·79–13·89μ); in *H. dictyoides* the mean width is 14·64–20·32μ, in the undescribed *Helminthosporium* on *Festuca* 15·88–18·27μ, and in another undescribed *Helminthosporium* 14·9–18·97μ. In *H. catenarium* mean spore width is 15·8μ (Graham, 1955) and 17·3μ (Nisikado). Spore length is also generally less in *H. phlei*. Forked spores are never seen in this fungus, though in *H. dictyoides* and the undescribed *Helminthosporium* spp. small numbers are frequently seen, especially in a moist chamber and cultures. On SPA *H. phlei* produces no pigment, *H. catenarium* from *Cinna arundinacea* produces a yellow one and *H. dictyoides* a pink one. *H. dictyoides* is the cause of net blotch on meadow fescue and is not pathogenic to timothy, while *H. phlei* produces necrotic and chlorotic blotches on timothy and the isolates used did not infect meadow fescue.

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EXPLANATION OF PLATES 17 AND 18

Plate 17

A–D. Conidia produced in a moist chamber on diseased seeds (Silwood material): A, from a seed with outer coat removed; B, five spores in a chain and a secondary spore; C, a chain of three spores; D, a chain of three spores with the first spore showing two successive second spores. × 120 and 480. E–G. Fructification produced in a moist chamber on seedlings (Silwood material): E, includes a long-branched conidiophore; F, includes two conidiophore-beak spores; G, long first spores. × 120.

Plate 18

H. Conidia from a diseased leaf kept for a few days in a moist chamber in Pennsylvania (Graham). × 120, 210 and 480.

I. Conidia from same material—leaves were again placed 7 days in a moist chamber in England. × 120 and 480.

J. Conidia produced in a moist chamber on a leaf from Silwood. × 120 and 480.

K. Fructifications of the fungus in a 7-day-old culture on Langeron glucose peptone agar. × 120.

L. Chlamydospores in a culture which shows a brown mycelium where the growth has completely stopped. × 120.

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A NOTE ON *WIESNERINA* (CYPHELLACEAE)

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(With 2 Text-figures)

Wiesnerina Hoehn. is a distinct genus from *Actiniceps* B. & Br., and possibly cyphellaceous. *W. secunda* Hoehn. is reduced to *W. horrida* Hoehn.

In showing that *Actiniceps* B. & Br. is a basidiomycete identical with *Dimorphocystis* Corner, Boedijn (1959) added *Wiesnerina* as a synonym. Donk (1959) was doubtful about *Wiesnerina*. It had been discussed by us in Singapore at the end of 1945 and excluded deliberately from clavarioid fungi. Through the courtesy of Dr M. M. Lamb, of the Farlow Herbarium, I have now been able to examine the type specimens of the two species ascribed to *Wiesnerina*. Their cystidia and hyphae are different from those of *Dimorphocystis*, and, as Boedijn figured for *Actiniceps* (fig. 2 of his paper) a cystidium like that of *Wiesnerina*, I have examined at the same time, through the courtesy of the Director of the Royal Botanic Gardens, Kew, the type of *Actiniceps*, *A. thwaitesii* B. & Br. I agree with Boedijn that *A. thwaitesii* is the same as *D. capitatus* Corner, but I could not find basidia on the Kew material and in view of the apparently conidial nature of the uncertain *A. cocos* Subramanian (1954), it is possible that *Actiniceps* represents a conidial state of *Dimorphocystis*: on the other hand *A. cocos* may be the conidial state of *Chaetotyphula* Corner. I agree, also, with Donk that *Wiesnerina* should not be reduced but maintained as a genus of very uncertain position.

WIESNERINA HOEHN.

Denkschr. Akad. Wiss. Wien, **83** (1907), 7; Boedijn, *Persoonia*, **1** (1959), 11–14.

W. horrida Hoehn. loc. cit.

W. secunda Hoehn. *S.B. Akad. Wiss. Wien*, Abt. 1, **121** (1912), 342, fig. 1; Donk, *Persoonia*, **1** (1959), 45–46.

Minute, gregarious, superficial, subdiscoid-obconic, – 370 μ wide (over-all), – 160 μ high (over-all), white, wholly minutely hispid-setulose, attached by a narrow base, but stemless, dried pale yellowish white and rather hard; hymenium covering the free distal surface, slightly convex.

On dead palm-leaves and tree-leaves; Brazil, Java.

Spores 5·5–6·5 × 3·7–4·2 μ, white, smooth, hyaline, ellipsoid, non-amyloid.

Basidia ? (2–4 sterigmata, Hoehn.).

Cystidia 90–140 × 9–19 μ, copious and projecting 10–50 μ from the whole surface, though shorter and fewer and more or less appressed on the sides of the fruit-body, fusiform to narrowly ventricose, the walls thickening – 5 μ, wholly minutely and regularly echinulate with fine spines – 1 μ long, often in rows and spaced 1·5–2 μ apart, not encrusted with crystals, the obtuse or subacute apex mostly thin-walled and smooth, the base tapered and smooth, the walls readily diffluent in dilute potash except for the echinulate pellicle.

Hyphae 2–2·5 μ thick, slightly thick-walled, monomitic, not encrusted, clamps ?

Collections: Hoehnel 605, auf baum-blatt, Sao Paulo, Alto da Serra, Brazil, May 1901, type of *W. horrida*; Hoehnel 625, palmenblattfiedern, Buitenzorg, Java, 1907–08, type of *W. secunda*.

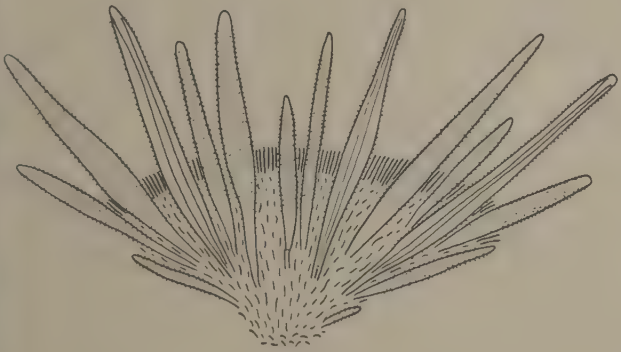

Fig. 1

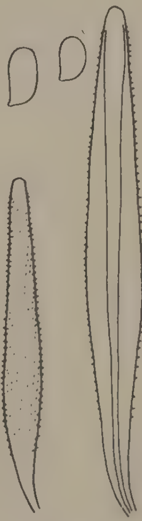

Fig. 2

Fig. 1. *W. horrida* Hoehn. Diagram of a median section through the fruit-body (type-collection). × 250.

Fig. 2. *W. horrida*. Cystidia, × 500. Spores, × 1000.

Apart from the habitat, I can see no difference between the two species. Both have the same spores and cystidia, which swell very characteristically in potash, as noted by Donk, and which show in the thick middle layer of the wall a slight amyloid bluish colour with Melzer's iodine. In neither could I detect basidia, but both had abundant basidiospores of characteristic shape. The hyphae were much collapsed, but there was no sign of the thick-walled skeletal hyphae distinctive of *Actiniceps*. The cystidia were of one kind only. There was no smooth hymenium, as in Cyphellaceae, but between the cystidia over the distal end of the fruit-body there were aggregated narrow hyphal ends, as in a hymenium, and among these were a few groups of four spores.

I conclude that this fungus is a basidiomycete. It is not *Actiniceps*, however, because it has only the one kind of cystidium and it is not dimitic in the manner of *Pterula*. Furthermore, the long cystidia of

Actiniceps are encrusted with excretory matter on smooth walls which are not echinulate as in *Wiesnerina* and are not diffluent in potash. Though Donk has excluded the genus from Cyphellaceae, yet the finely echinulate cystidia with diffluent walls resemble the external hyphae of some species of the family and, in the present artificial classification of basidiomycetes, I think that Cyphellaceae is the only family where the genus can be placed.

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DIMITIC SPECIES OF *RAMARIA* (CLAVARIACEAE)

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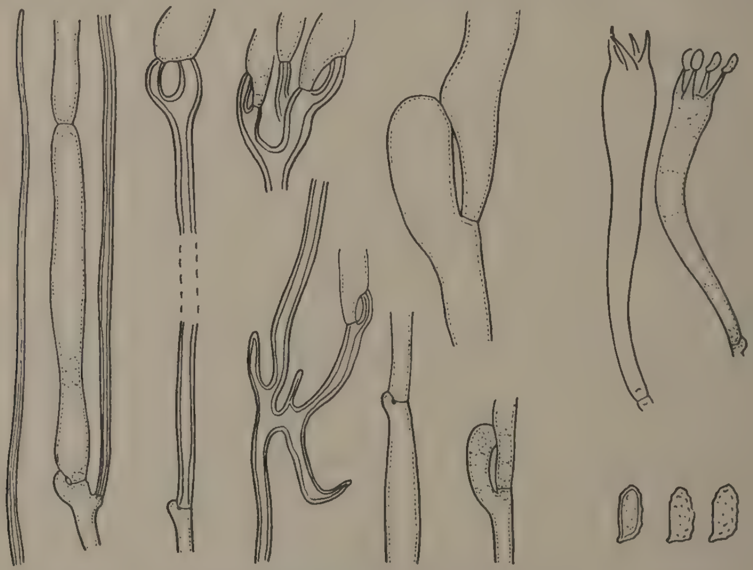
(With Plate 19 and 1 Text-figure)

Skeletal hyphae occur in the mycelium of *Ramaria gracilis* (Fr.) Quél., *R. moelleriana* (Bres. & Roum.) Corner, *R. stricta* (Fr.) Quél., and in collections referred to *R. acris* (Peck) Corner and *R. bourdotiana* Maire. Skeletal hyphae also occur in the fruit-bodies and mycelium of *R. flavoviridis* sp.nov. and *R. reticulata* (Berk. & Curt.) comb.nov., both of which are described. *R. apiculata* (Fr.) Donk is interpreted as a strictly monomitic species, as most of the genus. *R. pinicola* (Burt) comb.nov. is allied with *R. apiculata*. *R. albocinereum* (Pat.) Corner is reduced to *R. reticulata* var. *macrospora* Corner var.nov.

Skeletal hyphae, as unbranched thick-walled aseptate hyphae, have been recorded in the mycelium and rhizomorphs of *Ramaria stricta* (Fr.) Quél. (Corner, 1950). In the same work skeletal hyphae were recorded in the trama of *Lachnocladium reticulatum* Berk. & Curt. which, it was thought, might be a *Ramaria*. Two more collections now prove that this species is a dimitic *Ramaria*, and a remarkable ally, *R. flavoviridis*, has recently been discovered in India. Further investigation has shown the presence of the dimitic mycelium in *R. gracilis* (Fr.) Quél., *R. moelleriana* (Bres. & Roum.) Corner, which is very near to *R. stricta*, probably in the related *R. bourdotiana* Maire, and in a species tentatively called *R. acris* (Peck) Corner. In none of these, however, do the skeletal hyphae make the framework of the fruit-body, as in *Pterula* and the polypores. The situation is complicated and it seems opportune, in the rising interest in these fungi, to review it. We want to know if these hyphae in *Ramaria* are vestigial or incipient and it is probable that there are still unknown species which will help in the solution. Most of the species fall in the group *Stricta* of Corner's classification (1950, pp. 545, 548). The situation is presented in the following key:

Fruit-body dimitic, composed of thin-walled inflating generative hyphae with clamps and of relatively few skeletal hyphae, mostly as intercalary segments (150–1500 μ long) of the generative hyphae. Rhizomorphs also dimitic.
  Skeletal hyphae 3–5 μ wide, sparse in the rhizomorphs. *R. flavoviridis*
  Skeletal hyphae 1–3·5 μ wide, composing most of the rhizomorphs. *R. reticulata*
Fruit-body monomitic with inflating clamped hyphae mostly with thickened walls (1–2 μ).
  Rhizomorphs and mycelial subiculum dimitic.
    Rhizomorphs with copious narrow skeletal hyphae 1·5–3 μ wide, of limited growth with tapering filiform ends. *R. bourdotiana*, *R. gracilis*, *R. moelleriana*, *R. stricta*
    Rhizomorphs composed mainly of thin-walled hyphae with sparse skeletal hyphae 3–5 μ wide as intercalary segments. *R. acris*
  Rhizomorphs monomitic. *R. apiculata*, *R. pinicola*

The fruit-bodies of *R. flavoviridis* and *R. reticulata* are supported by the turgidity of the inflated, intertwined, thin-walled hyphae, which never become as thick-walled as those of the *R. stricta*-group, though the walls become firmer at the base of the branches. Among these hyphae occur, mainly as intercalary segments, the sparse skeletals which do not seem to contribute mechanically to the construction: thus, they are specialized intermittent products of the thin-walled hyphae developing at the tips of the branches and characterized by the thickening of the wall without inflation of the cell. Though sparse, these skeletal hyphae are conspicuous in any fragment of the trama. *Amylaria* (Corner, 1955) has much the same structure but its thin-walled hyphae scarcely inflate and the skeletals are copious: this genus differs profoundly in the amyloid spores.



Text-fig. 1. *Ramaria flavoviridis* sp.nov. Spores and basidia, × 1150; skeletal and generative hyphae, × 500.

The fruit-bodies of the *R. stricta* group are supported not only by the turgidity of the monomitic hyphae but by the strong thickening of the walls of most, if not all, of them. The dimitic state in the mycelium can have no truly skeletal value. *R. acris* is intermediate in having the fruit-body constructed as in *R. stricta* but the rhizomorphs as in *R. flavoviridis*. In *R. reticulata* the rhizomorphs have copious narrow skeletal hyphae of limited growth as in *R. stricta*. Thus, on mycelial characters, *R. acris* agrees with *R. flavoviridis*, and *R. reticulata* with *R. stricta*, but on fruit-body characters, *R. acris* agrees with *R. stricta* in contrast to *R. reticulata* and *R. flavoviridis*.

R. apiculata (Fr.) Donk and *R. pinicola* (Burt) Corner, which is redescribed in this paper, are very like *R. stricta* in form, colour, spores, and hyphae but their rhizomorphs and mycelial subiculum are monomitic, composed of narrow, slightly thick-walled, clamped hyphae with scattered ampulliform swellings. This is the state in all other species of *Ramaria* which I have studied. Thus, the construction of the rhizomorph is a valuable help in identifying dried material. The species of *Lentaria* which approach *Ramaria* have a similar monomitic mycelium.

The temptation is to make a new taxon for *R. flavoviridis* and *R. reticulata*, but we must await fuller exploration of the tropical fungi. Their spores are scarcely distinguishable from those of the *R. stricta* group, and, though they tend to have flattened branching, this occurs also in *R. gracilis*, of which *R. palmata* is merely the state with such flattened branching. This character generally accompanies lack of inflation of the hyphae, as in *Amylaria*, *Lachnocladium*, *Pterula* and *Thelephora*, but the hyphae inflate strongly in *R. flavoviridis*. Skeletal hyphae, on the other hand, commonly accompany the lignicolous habit, though there are exceptions, and the dimitic group of *Ramaria* is that most closely connected with this habit. The immediate inference is the evolution of skeletal hyphae along with the lignicolous habit and loss of inflation of the hyphae, parallel to the process of *Polyporus* and *Stereum*.

Ramaria acris (Peck) Corner comb.nov.

Clavaria acris Peck, *Rep. N.Y. St. Mus.* **54**, 155, 1901; Corner (1950), 556, sub *R. apiculata*

Spores 6·5–8·5 × 3·5–5 μ, minutely subechinulately rough.

Hyphae of trama < 10 μ wide, with ampulliform swellings < 20 μ wide, the walls thickening < 2·5 μ, clamped.

Mycelial hyphae 2–6 μ wide, with scattered ampulliform swellings, the walls thin or slightly thickened, clamped: skeletal hyphae 3–5 μ wide, scattered, with glairy walls 1–1·5 μ thick, unbranched, mostly as long intercalary segments.

Collections. J. R. Weir 15478, Idaho, pr. Eagle, Shoshoun Co., leg. A. S. Rhoades, on the side of a rotten coniferous log and in neighbouring humus, 'pinkish all over': J. B. Flett s.n., Washington, Bremerton, 15 Oct. 1933 (mixed with *Clavulina cristata*): Mo. Bot. Gdn Herb. no. 151040, leg. L. R. Hesler no. 11584, N.C., Great Smoky Mts. National Park, on rotten hemlock wood (det. *Clavaria apiculata*): Mo. Bot. Gdn Herb. no. 57459, leg. Ann Hibbard, Mass., West Roxburg, on wood, 'turns brown on bruising, taste acrid, not bitter': Herb. Krypt. Mus. Nat. Prague 168341, Slovenia, sub Abiete (possibly only *R. gracilis*).

Ramaria apiculata (Fr.) Donk

Corner (1950), 555; Malençon, *Bull. Soc. mycol. Fr.* **73** (1957), 291; Pilát, *Acta Mus. nat. Prag.* **14** (1958), 166, t. 17b, 18a; Pilát, *Čes. Mykol.* **13** (1959), 75, t. 1.

The monomitic mycelium distinguishes this from *R. stricta*. I have not been able to study living material. I refer here the following collections:

J. R. Weir 15943, Idaho, Priest River: Weir 15967, same locality (some collections are *R. apiculata*, others *R. pinicola*): Mo. Bot. Gdn Herb. no.

57460, leg. Ann Hibbard, Vt. Pomfret: Mo. Bot. Gdn Herb. no. 56534, leg. E. A. Bessey no. 23, Mich., Gogetic Co.: Lloyd Cat. no. 19098, leg. C. G. Lloyd, Sweden, Drottningholm: Lloyd Cat. no. 41840, leg. S. R. Kashyap no. 23, Pakistan, Lahore: S. Ahmad no. 4508, 4733, Pakistan, Murree River: K. S. Thind no. 69, India, Simla.

Ramaria bourdotiana Maire

Corner (1950), 563

I refer here Lloyd Cat. no. 19133 (leg. M. Barbier, France, Côte d'Or). It has been referred to *R. stricta* but it has smaller spores, 6–7·5 × 3–3·7 μ (finely rough to subverruculose). The hyphae agree with *R. stricta*, not with *R. crispula*, which has the normal monomitic mycelium.

Ramaria flavoviridis Corner & Thind sp.nov. Text-fig. 1, Pl 19.

Ad 17 × 7 cm., gregaria v. caespitosa, trunco subnullo e basi multiramosa, carnosa, laete viridis dein flavoviridis, apicibus concoloribus v. albidulis, rhizomorphis gracilibus albis copiosis praedita; ramis polychotomis, superne dichotomis et plus minus compressis; carne alba insipida, Anethisoa odore.

Ad terram in silvis (Quercus–Cedrus), India, Dalhousie, Bakrota, Thind n. 128 (typus in herb. Punjab University).

Sporae 6–8·3 × 3–3·7 μ, brunneolo-ochraceae, ellipsoideae, subverruculosae v. subechinulatae. Hyphae in tramis receptaculorum rhizomorphisque dimiticae; skeletales 3–5 μ latae, tunicis 0·5–1 (–1·5) μ crassis, sparsae sed conspicuae, vix ramosae, aseptatae, ad 1500 μ longae, apicibus filiformibus elongatis 1 μ latis vel ut segmenta intercalaria; hyphae tenuitunicatae 2·5–9 μ latae, hinc inde ad 17 μ, copiosae, fibulatae, cellulis ad 140 μ longis.

Up to 17 × 7 cm., gregarious or caespitose, erect, much branched, fleshy, light green when young, becoming yellowish green, the tips concolorous to whitish, drying pale brown, attached by slender white rhizomorphs, trunk absent, branching from the base: branches polychotomous below, dichotomous above, the branching generally flattened and in one plane but the planes alternating (when dried 1–1·5 mm. wide, dilated 2–3·5 mm. below the axils), tips acute or obtuse: flesh white, unchanging: taste inparticular: smell of the fruits of *Anethum soa*.

On the ground in mixed Quercus–Cedrus forest: India, Dalhousie, Bakrota, leg. Thind no. 128 (Type), 30 Aug. 1958.

Spores 6–8·3 × 3–3·7 μ, light brown to ochraceous, ellipsoid, obscurely verruculose to almost subechinulate, the apiculus < 0·7 μ long, not amyloid. Basidia 4-spored. Hymenium thickening < 250 μ, amphigenous. Cystidia none.

Hyphae dimitic in the flesh and rhizomorphs. Generative hyphae 2·5–9 μ wide, with swellings < 17 μ wide, composing most of the flesh, thin-walled, clamped, the cells < 140 μ long, often with the clamps enlarged. Skeletal hyphae 3–5 μ wide, the walls 0·5–1 (–1·5) μ thick, relatively sparse but conspicuous, aseptate, rarely branched, longitudinal, < 1500 μ long, ending blindly in the flesh with long tapering subacute ends 1 μ wide or, mostly, as intercalary parts of generative hyphae and, then, often with a few short branches terminated with thick-walled clamps, occasionally anastomosing.

This differs from *R. reticulata* in the large size and, probably, the yellow-green colour of the fruit-body, the narrower spores, and the wider generative and skeletal hyphae. Perhaps the soil habitat is different, but it may have grown from buried wood. It is one of the more interesting discoveries among Clavarias in recent years. Beware *Amylaria himalayensis* Corner (1955) with similar flattened branching and dimitic hyphae, but with amyloid spores.

Ramaria moelleriana (Bres. & Roum.) Corner, *Darwiniana, B. Aires*, **11** (1957), 202.

Ramaria pinicola (Burt) Corner comb.nov.

Clavaria pinicola Burt, *Ann. Mo. bot. Gdn*, **9** (1922), 25, pl. 5, fig. 32; Doty, Clav. Pac. N.W. (1944), 44; Corner (1950), 556, sub *R. apiculata*; ? *C. stillingeri* Coker, *J. Elisha Mitchell sci. Soc.* **42** (1927), 251, pl. 47; Corner (1950), 557, sub *R. apiculata.*

Up to 6 cm. high, gregarious to caespitose, with few to many branches, buff pink to greyish drab, tips pale lemon yellow, becoming brownish from the base: stems $< 25 \times 2$–8 mm., often fastigiate; branches dichotomous; taste slightly bitter.

On humus and trunks in coniferous forest, especially of *Pinus*, U.S.A. (Montana, Idaho, Washington, Oregon, California).

Spores (8·5–) 9·5–11 (–11·5) × (4–) 4·7–6 (–6·5) μ, ochraceous, smooth, bluntly ellipsoid.

Hyphae of trama clamped, the walls -2μ thick. Mycelial hyphae monomitic, 2–4μ wide, with ampulliform swellings $< 12\mu$ wide, the walls thin or slightly thickened, the outer hyphae of the rhizomorphs incrusted with granular and crystalline matter.

Collections. J. R. Weir 15510, 15514, 15542, 15543, 15674, 15906, 15941, 15942, 15967, 16724, 16735, 16946 (*typus*), 16948, 16952, 16953, 16954, 16955, 16958, 16960: W. B. Cooke 21437, 22178, 22470, 22591; J. B. Flett 129, 179; E. E. Morse 185 (mixed with *Clavulina cristata*); F. H. Rose 3416, 3424, 3643, 3877; Shear and Henderson 4520 (Mo. Bot. Gdn Herb. no. 55926).

These numerous collections show that this is a distinct species from *R. apiculata*, though macroscopically very similar. The wide smooth spores are characteristic, but they raise the question whether *R. pinicola* is not identical with the European *R. condensata* (Fr.) Quél, *R. karstenii* (Sacc. & Syd.) Corner, and *R. spinulosa* (Fr.) Quél.

Ramaria reticulata (Berk. & Curt.) Corner comb.nov.

Lachnocladium reticulatum Berk. & Curt., *J. Linn. Soc.* (*Bot.*), **15** (1876), 388; Corner (1950), 436.

Up to 7 cm. high, caespitose with many narrow fastigiate stems, dried pallid brown or fulvous, with slender white rhizomorphs; branches (dried) 0·3–0·5 mm. wide below, trichotomous or dichotomous below, dichotomous distally, with flattened branching, the axils slightly dilated and

1–1·5 mm. wide, crowded so as to appear reticulate, 'apicibus albis' (Rick); hymenium often unilateral.

On rotten wood, Brazil.

Spores 6–7·5 × 3·7–5·3 μ, pale ochraceous, broadly pip-shaped, slightly rough or subrugulose (not echinulate or verrucose), with prominent apiculus 1–1·5 μ long, not amyloid.

Basidia 4-spored. Hymenium thickening slightly. Cystidia none.

Hyphae dimitic both in the trama and rhizomorphs; generative hyphae 2·5–8 μ wide, thin-walled, clamped, more or less inflating, copious; skeletal hyphae 1–3·5 μ wide, unbranched, the walls 0·5–1 μ thick, aseptate, flexuous, longitudinal ending blindly in the tissue or as elongate intercalary segments < 600 μ long, much less abundant than the generative hyphae in the trama, but composing most of the tissue of the rhizomorphs.

Collections. Traill 127, Rio Jurua, type in herb. Kew: Rick s.n., Rio Grande do Sul, Nova Petropolis, April 1920.

var. **macrospora** Corner var.nov.

Lachnocladium albocinereum Pat., *J. Bot., Paris* (1889), p. 33.
Ramaria albocinereum (Pat.) Corner (1950), p. 555.

Sporae 7·5–9·5 × 4·5–5·3 μ, verrucis applanatis 1 μ elevatis ornatae.

Ad lignum putridum, French Guiana (Melinon 100, Maroni: L. albocinerei typus in herb. Patouillard, Farlow Herb.).

Except for the longer and distinctly verrucose spores, I can see no difference from var. *reticulata.*

Grateful acknowledgement is made for the loan of herbarium-material to the Curator of the Farlow Herbarium, the Director of the Missouri Botanical Garden, and to the Director of the U.S. National Mycological Collections, U.S. Department of Agriculture, Beltsville, Md.

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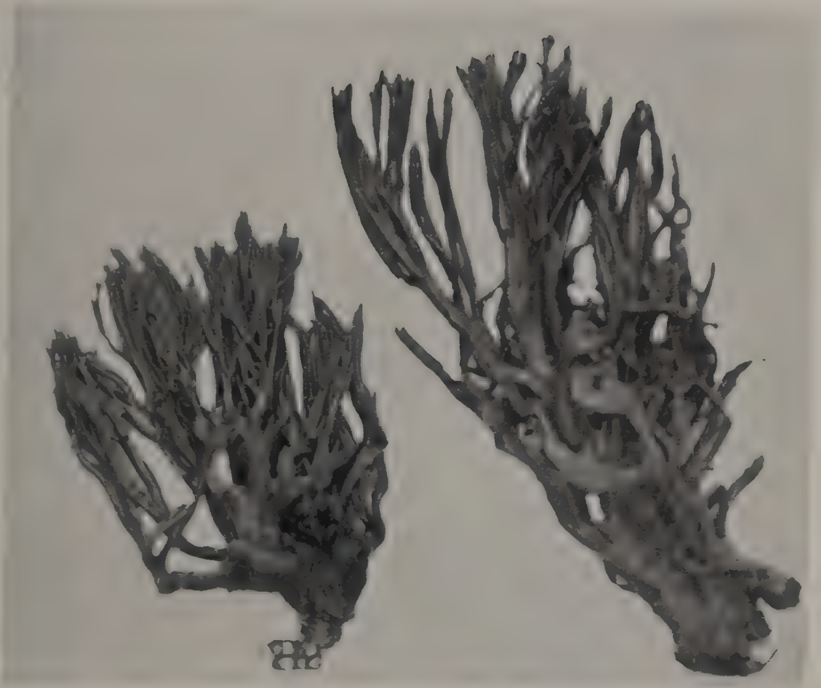
EXPLANATION OF PLATE 19

Ramaria flavoviridis sp.nov. Fruit-bodies preserved in alcohol-formalin. × 1.

(*Accepted for publication* 11 *July* 1960)



(*Facing p.* 238)

Trans. Brit. mycol. Soc. **44** (1), 239–242 (1961).

A NEW TECHNIQUE FOR ISOLATING SPORES OF YEAST (*SACCHAROMYCES CEREVISIAE*)

By B. S. COX and E. A. BEVAN

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(With 2 Text-figures)

A new technique for the dissection of yeast asci is described. It depends upon the use of 'Cellophane' as a surface for dissection, and the incubation of the asci on nutrient medium prior to dissection to facilitate their rupture.

Methods for the dissection of yeast asci already described have two main disadvantages: first, the spores may be damaged when the ascus is ruptured, thus reducing their viability; and secondly, the spores have to be transferred over comparatively long distances involving multiplication of operations and consequent loss of time (Winge & Laustsen, 1937; Lindegren, 1949). Further complications arise when, as is commonly the procedure, two micro-instruments are used during the operations: a micro-needle for the dissection of the ascus on the underside of a glass coverslip, and a micro-loop for the transference of asci before dissection, and of spores after dissection, into separate nutrient droplets placed on the same or different coverslips.

The technique to be described here tends to eliminate these disadvantages. It depends, first, on the use of 'Cellophane' as a surface for dissection. This does away with the necessity of transferring each spore individually into separate nutrient droplets. All can be lifted together with the Cellophane and placed on nutrient agar. Water and nutrients can diffuse through the Cellophane, and so the spores can grow on its surface perfectly well. Secondly, we have found that asci are very much easier to break, and often rupture of their own accord, if they are incubated on nutrient agar prior to their dissection.

MATERIALS AND APPARATUS

Cellophane of grade P.T. 400 (kindly supplied by British Cellophane Ltd., Bridgwater, Somerset) is cut into slips $\frac{1}{2} \times \frac{7}{8}$ in. Five cuts are made in each slip, rather more than half-way across its breadth (Fig. 1).

Micro-instruments are made with the de Fonbrune micro-forge. A micro-loop is only used for transferring asci from the stock suspension to the Cellophane, and a micro-needle for both the dissection of the ascus and the sorting out of spores after dissection. The dimensions of the micro-loop are shown in Fig. 1. Micro-manipulation is carried out under the low power of the microscope in a simple humidity chamber, using a Singer micromanipulator (Barer & Saunders-Singer, 1948).

Isolation and pre-incubation of asci

A slip of Cellophane is placed on a 1 in. square coverglass. A small hanging drop of a stock suspension of asci is then placed 0·5 cm. or more away from the Cellophane slip. Five asci are transferred from the hanging drop to the Cellophane slip with the micro-loop. These asci are easier to find later if each is placed near the end of a cut in the Cellophane. Cellophane slips with asci uppermost are placed on nutrient agar in a Petri dish and incubated at 28° C. for approximately 2½–3 hr., by which time the swelling of the spores would have ruptured or weakened the ascus wall.

The best length of time for such partial germination of asci varies according to the yeast strain. It is important not to incubate too long, otherwise the spores may start budding or mating or both. Linear asci usually burst more easily than lozenge-shaped ones and are altogether easier to handle. If it is necessary to maintain the order of the spores in a linear ascus, as, for example, when mapping the distance of genes from the centromere, it is better to pre-incubate for a shorter period (2–2½ hr.) to ensure that none of the asci rupture.

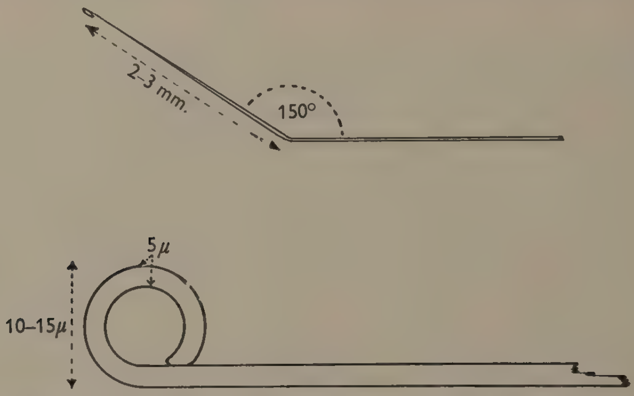

Fig. 1. Diagrams illustrating the dimensions of the micro-loop used in isolating the asci.

Dissection of asci and separation of spores

The spores are dissected from each ascus under low power of the microscope with a micro-needle. This can usually be done without removing the Cellophane from the Petri dish. The dish is placed on the microscope stage and the micro-needle lowered onto the Cellophane slip. The needle must be mounted in the manipulator so that its shaft does not hit the edge of the dish during manipulation. The spores from each ascus are separated and spaced out on the Cellophane to the limits of the lower-power field. The procedure is illustrated diagrammatically in Fig. 1.

To maintain the order of the spores in a linear ascus it is necessary to transfer the Cellophane back to a coverglass and invert it over the humidity chamber. The ascus is then easily dissected under the high power, and the spores are separated under the low power of a compound microscope. The micro-colonies formed by the spores are subcultured 24 hr. later; if they are left much longer they may grow into each other. Owing to their proximity, a dissection microscope is necessary when subculturing. We have found it helpful to use a clamp for steadying the holder of the tungsten wire needle used for their subculture.

Moisture conditions are important. If the Cellophane has too much surface moisture when the asci are transferred to it from the stock suspension, they may float away and be lost; if it is too dry following the ascus pre-incubation period, the spores are difficult to move around on its surface. This latter problem does not arise if dissection and separation are done in a Petri dish. But if the Cellophane is transferred to a coverglass for dissection, it is best to moisten the coverglass first and to take each ascus separately by tearing the Cellophane into complete strips along the cuts already made.

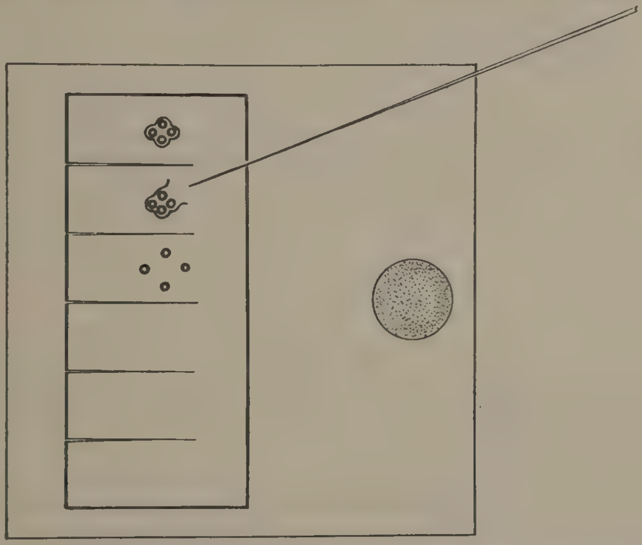

Fig. 2. Diagram illustrating a Cellophane slip and the three stages in dissection of an ascus. Dissection may be carried out on a coverglass, as illustrated, or on a Petri dish. A hanging-drop of stock suspension of asci is shown on the right of the coverglass.

EFFICIENCY OF THE TECHNIQUE

In the course of our work approximately sixty genetically different diploid strains have been dissected by Lindegren's method (1949). This involves rupturing the asci by rolling them on the underside of a coverglass with a micro-needle and transferring each spore to a separate nutrient droplet. The germination of these strains is usually about 40%, and lower percentages were common. Only in four of these strains has the germination exceeded 75 %.

Four strains have been dissected by our method so far. They have given germination percentages of 95, 50, 83, and 95, respectively. Up to the present a full comparison of our method with that of Lindegren has been carried out with one strain only. Using Cellophane, both with and without pre-incubation of asci, the asci of this strain gave 95 % germination (30 asci); Lindegren's method gave 60 % germination (80 asci).

Further, our method cuts down the time needed for dissection. Once the asci have been isolated and pre-incubated, up to twenty per hour may be dissected and their spores separated. The number which can be dissected in a given time is generally limited by the relative proportions of asci and vegetative cells in the stock suspension, which varies with the strain. Sometimes it takes 2 min. and at other times ½ hr. to find and isolate five asci.

There is also a considerable saving in the time taken for preparing the apparatus. Cellophane slips and coverglasses may be sterilized in bulk and used as required. After that, all that is needed are a number of dishes of nutrient agar and the setting up of the micromanipulation apparatus.

One of us (B.S.C.) is indebted to the Department of Scientific and Industrial Research for a Research Studentship.

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(*Accepted for publication* 9 *April* 1960)

ADDENDUM

Since this manuscript was submitted for publication, Hawthorne & Mortimer (*Genetics* **45**, 1085–1122, 1960) have reported studies on gene mapping in which they facilitated the breakdown of the ascus wall by preincubation in an enzyme obtained from snail gut. We have found this method of rupturing the ascus wall as good as (and for some yeast strains even better than) our method of preincubation on nutrient medium. For maintaining the order of spores in linear asci, however, we find that preincubation on nutrient medium results in less rearrangement of the spores prior to their dissection.

Trans. Brit. mycol. Soc. **44** (2), 243–252 (1961).

SPECIES OF *MORTIERELLA* FROM A SALT MARSH

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(With Plate 20 and 3 Text-figures)

The occurrence of six species of *Mortierella* in a salt marsh habitat is recorded, descriptions of certain isolates are given, and the taxonomic relationships of some species are discussed.

During investigations by one of us (G. J. F. P.) of the fungus flora of a developing salt marsh, several species of *Mortierella* were noted, accounting for about 6·6 % of the total number of isolations made. Investigations too numerous to quote have shown *Mortierella* to be well represented in soils of almost every type, but the only previous record from a salt marsh soil seems to be a report by Bayliss Elliott (1930) of a variant of *M. pusilla*.

THE HABITAT

The isolations were made from samples collected from mud flats at Gibraltar Point, Lincs. (National Grid Ref. TF 555576). Mud uncolonized by higher plants was taken from well below the *Salicornia* zone, and further samples were obtained from each of the major vegetation zones.

The flats are regularly inundated by the sea. The lower reaches remain water saturated, with a water content of up to 65 % when newly exposed; the uppermost regions, though covered by spring tides, dry out, to a degree determined by the prevailing weather conditions, during neap tides. A water content value of 12 % of the oven dry weight has been obtained at such a time from the top of the *Salicornia* zone.

The reaction of the mud is pH 7·6–8·2 (glass electrode determinations). The chloride content, expressed as g./kg. soil water, determined by the chromate titration method, was usually near 18, with extremes of 9 in the uncolonized mud and 42 in the upper *Salicornia* zone during a neap tide period.

The organic matter content of the uncolonized mud is low, the Walkley & Black value for organic C being about 0·9 %. Total N, determined by the Kjeldahl method, is about 0·07 %. In the surface layer (0–5 cm. deep) of the *Salicornia* zone the values are 2·2 % for organic C and 0·23 % for total N.

Conditions tend to be anaerobic, especially where there is a high proportion of fine silt forming patches of sticky mud where little water movement can occur, and the activities of sulphate-reducing bacteria cause blackening. Where blown sand has been deposited and mixed with the silt, more aerobic conditions are suggested by reddish brown patches where iron sulphide has been re-oxidized. The mud round plant roots is often black, possibly because of the reducing activities of associated micro-organisms.

COLLECTION AND ISOLATION

Samples of mud were obtained with a borer of 5 cm. diam., normally to a depth of 8–10 cm. Cores were subsequently cut open longitudinally and inocula of mud from the centre were plated out by Warcup's technique (1950). From colonized mud cores were taken so that each included most of the roots of an individual plant. These were used to sample the rhizosphere flora by plating out portions of mud in contact with the roots.

Sporangiophores of *Mortierella* species were observed in the plates and representative specimens isolated. Later, a specific search for these species was instituted, using plates inoculated with small portions of mud and fragments of plant debris placed on the surface of the agar (Waksman, 1916). These were examined after 2 or 3 days, when rapidly growing *Mortierella* colonies were sufficiently recognizable to allow hyphal tip isolation. The plates were kept for a week or longer, but no further *Mortierella* species were observed.

Early isolations were made on 2% plain agar, but later a simple extract of salt marsh mud in distilled water solidified with agar (MEA) was used. This was prepared by steaming 1 kg. mud/l. of water for 1 hr., straining through coarse filter-paper (Green's 904), making up to volume and adding agar. The reaction was approximately pH 7·5 and did not require adjustment.

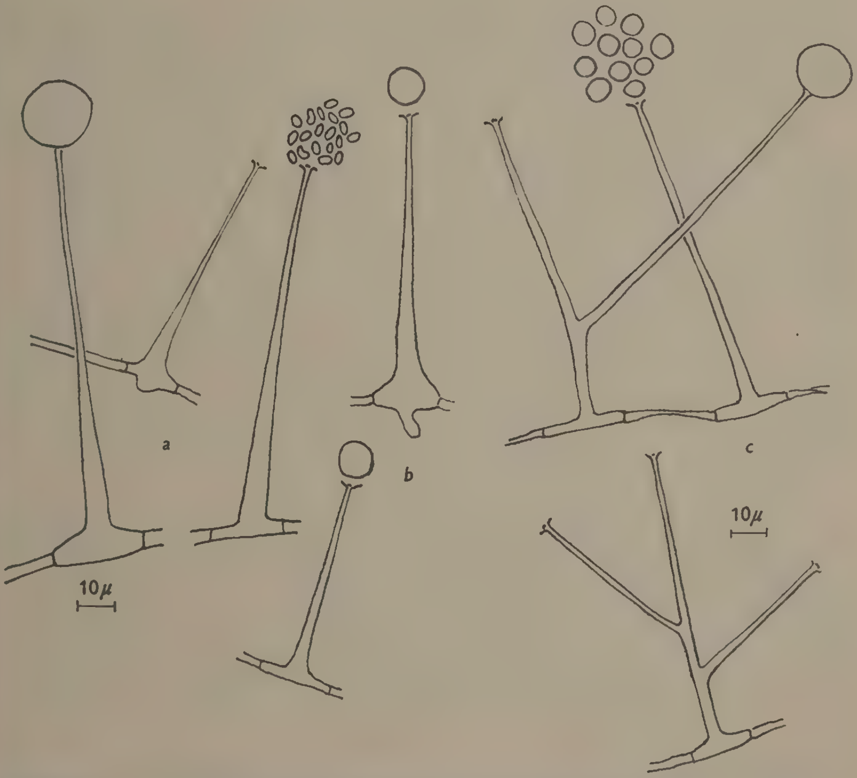
Cultures were grown on 2% malt-extract, potato-dextrose and maize-extract agars for comparison with descriptions given by Linneman (1941) and other authors. Soil-extract agar (SEA), which has been found previously to be very satisfactory for species of *Mortierella*, was also used; it encourages the production of sporangiophores which are not obscured, as they are on richer media, by over-abundant vegetative mycelium. The sporangiophores may be more luxuriantly branched than on richer media, but are otherwise similar in form and dimensions. The dimensions of sporangia and spores are not affected, and those species which normally form chlamydospores produce them abundantly on SEA; MEA was even more satisfactory. A variant of this medium containing 0·1% $(NH_4)_2HPO_4$ and 0·5% glucose produced more abundant and characteristic mycelial growth, but did not proportionately increase sporulation. The addition of yeast extract did not improve the medium for species of *Mortierella*.

Measurements were made by eyepiece micrometer on material freshly mounted in 0·9% saline solution. Small spores (under 5μ) were measured from camera lucida drawings of at least fifty spores after comparison with micrometer measurements.

SPECIES ISOLATED

MORTIERELLA ALPINA Peyronel (1913) was the commonest *Mortierella* species in the salt marsh, forming about 5% of the total number of fungus isolations (over 2000). It has been found at all sites examined, relatively rarely in the lowest areas (about 2% of the isolations), but increasing in abundance with increasing colonization by higher plants, to a maximum of about 16% in the mature *Puccinellia maritima* sward.

Isolates M 144 from the bare mud, M 153 from a decaying *Salicornia* fragment and M 160 from the *Spartina* zone were examined in detail and found to correspond closely with the description of the species given by Linneman (1941) except that no mature zygospores have been seen. Linneman reported that almost all her isolates of this species formed numerous anastomoses, which she regarded as gametangial stages, and abundant zygospores. Our isolates formed anastomoses and on some media produced conspicuous hyphal knots, but on dissection these were found to consist of interwoven hyphae containing abundant oil, with no sign of a zygospore. Possibly these hyphal knots, commonly reported in *Mortierella*, represent abortive attempts at zygospore formation.



Text-fig. 1. *a*, *b*, *M. alpina*, sporangiophores with normal and monosporous sporangia; *c*, *M. minutissima* sporangiophores and spores.

In some subcultures isolate M 144 produced, in addition to normal sporangia, others in which the contents failed to divide into the typical ellipsoidal or reniform spores, 2–5μ long, but formed a single more or less globose 'monospore' 8–10 μ diam. (Text-fig. 1 *b*). Similar structures occur in other isolates of *M. alpina*, and were described by Björling (1936) for his *M. thaxteri*, which also produced hyphal knots but no zygospores, and which Linneman regarded as synonymous with *M. alpina*.

M. MINUTISSIMA van Tieghem (1876) has been found in all zones except the bare mud and comprised about 1·3 % of all fungus isolations. Four strains from the *Salicornia* zone (M 149, M 150, M 151, M 152) were examined in detail and found to agree with Linneman's description of the species, except for slight differences in mycelial habit and in possessing the typical garlic-like smell of the genus. The original description by van Tieghem is very brief, and Linneman stated that she found the species very variable. In our cultures the substrate mycelium on malt agar forms a rosette of rounded lobes, variable in size but usually about 3–5 mm. across (Pl. 20, Fig. 3). The aerial mycelium is moderately well developed, with stolons ending in rhizoids and bearing the delicate sporangiophores. These are 50–180 μ long, but usually less than 100 μ, and taper from 4 to 6 μ wide at the base to 1·5–3 μ at the apex. They may be simple or with sparse cymose branches of variable length arising usually from the lower part of the main axis (Text-fig. 1 *c*). The sporangia are 15–20 μ diam. (10–15 μ according to Linneman, about 20 μ in van Tieghem's illustration) and have a readily diffluent wall which leaves a small but distinct collar. Most sporangia contain about ten thin-walled, round or slightly oval spores, 4–10 μ diam., about 80 % measuring 5–7 μ (Text-fig. 1 *c*). This agrees with Linneman's measurements, though van Tieghem gives 8–10 μ. No chlamydospores or zygospores have been seen.

M. SPINOSA Linneman (1936) was isolated twice from the *Salicornia* zone, and the isolates (M 157 and M 158) agree closely with the descriptions given by Linneman (1936, 1941). The spores are usually oval to ellipsoidal, as in some of Linneman's strains. Young sporangiophores may resemble those of *M. polycephala* Coemans, but arise singly, not in groups. Later, the much longer, upward-curving branches, arising from the upper part of the main axis and forming a corymbose, often complex structure, are characteristic (Text-fig. 2 *a*, *b*).

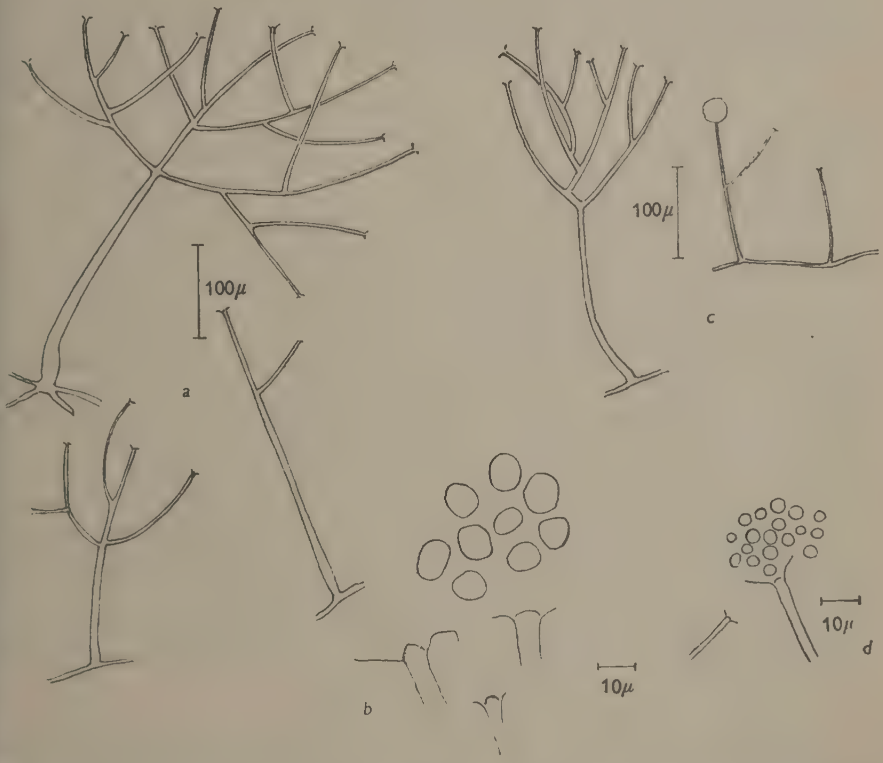
M. PARVISPORA Linneman (1941) was isolated once from the *Spartina* zone. The specimen, M 159, is in good agreement with the original description and with a culture from another source, identified by Dr Linneman as *M. parvispora* (Text-fig. 2 *c*, *d*).

M. POLYCEPHALA Coemans (1863). Two almost identical isolates, M 147 from the *Spartina* zone and M 148 from the *Salicornia* zone, are here referred to this species, which has seldom been reported from soil. Like other closely related species, it is more characteristic of rich organic substrates such as dung or decaying basidiomycete fructifications.

On malt agar the substrate mycelium may form two or three broad irregular zones at first, becoming lobed as the colony spreads, or may form a rosette of rounded lobes, variable in size but usually 1–2 cm. across

(Pl. 20, figs. 1, 2). Aerial mycelium is not very profuse. Abundant stylospore production may make the colony appear slightly powdery, while tufts of sporangiophores give a flocculent appearance. On MEA mycelial growth is sparse but stylospores and conspicuous tufts of sporangiophores are formed.

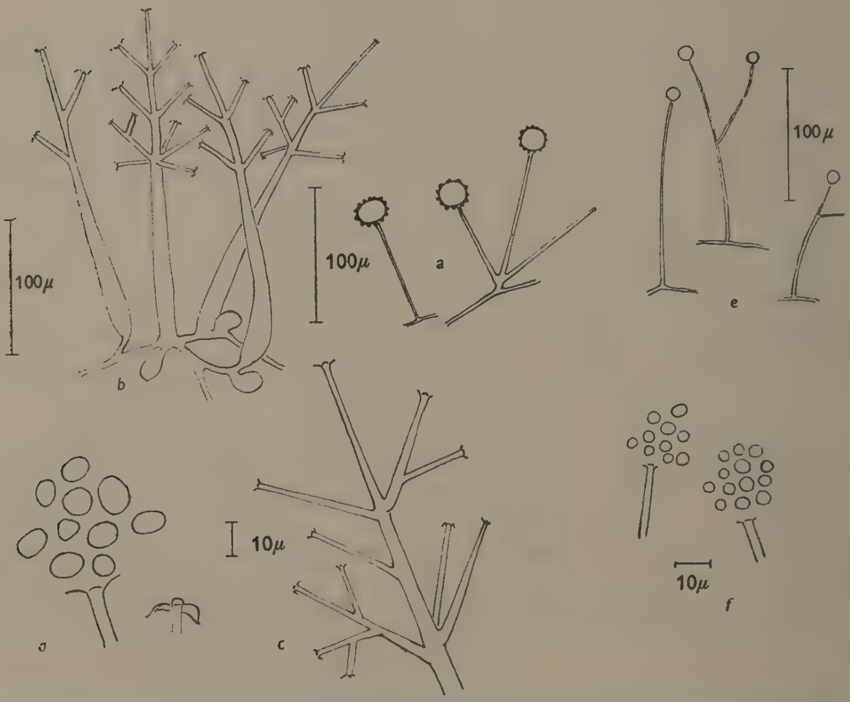
The stylospores are borne on slender stalks of uniform diameter (about 2μ), 15–75μ long, simple or branched, arising from submerged or aerial hyphae. They may also be produced below the surface of the agar, where they can be distinguished by their globose or subglobose form and thick, verrucose walls from the smaller, more or less ellipsoidal, smooth-walled, intercalary chlamydospores. Stylospores range from 10 × 12 to 20 × 24μ, the majority measuring 14–16 × 16–18μ (Text-fig. 3*a*).



Text-fig. 2. *a, b, M. spinosa*, sporangiophores, spores; *c, d, M. parvispora*, sporangiophores, spores.

The sporangiophores arise from stolons or from prostrate aerial hyphae and may have a few short rhizoids. They appear singly at first, but others soon arise in close proximity, so that within a few days the culture is dotted with clusters of up to 6 or 7 sporangiophores arising from the same rhizoidal complex, as described by van Tieghem & le Monnier (1873) for

M. polycephala, by Dauphin (1908) for this species and for *M. raphani* Dauphin and *M. canina*, and by Bachmann (1900) for *M. vantieghemi*. The sporangiophores are 200–300μ long and 10–20μ broad at the base, tapering at first sharply and then more gradually to about 2–3μ at the apex. When they arise from the tips of rhizoidal branches they are narrow at the base, swelling sharply to maximum width before tapering to the apex, but when they arise laterally on hyphae at or above the surface of the medium this basal constriction is absent. Both conditions may be seen in the same group of sporangiophores (Text-fig. 3*b*), and the separation by Vuillemin (1918) of *M. lemonnieri* from *M. polycephala* mainly on this criterion, is clearly not justified. Branching is confined to the upper part of the main axis, where up to four or five whorls of primary branches may be produced. The first whorl often consists of three to six branches, each about 30–40μ long, succeeding whorls consisting of fewer, shorter branches. These are usually almost uniform in diameter (2–4μ), but are sometimes slightly swollen at the base, as in *M. raphani*. In living material they tend to stand out stiffly from the main axis at an angle of about 45°, but this feature may be obscured in mounted specimens. Secondary and occasionally tertiary branching may occur. The terminal sporangium is usually 30–40μ diam. and contains more than twenty spores, lateral



Text-fig. 3. *a–d*, *M. polycephala*, stylospores, group of sporangiophores, detail of branching, spores; *e*, *f*, *M. turficola*, sporangiophores, sporangia, spores.

sporangia being smaller (12–20μ) with fewer spores. The sporangial wall dissolves readily, leaving a collar which may be broad and often reflexed, or smaller and less conspicuous. The transverse septum is slightly convex. The spores are smooth, thin-walled, round to oval or slightly irregular, and range from 5 to 14μ diam., 70% or more measuring 8–10 × 7–9μ (Text-fig. 3*d*).

The occurrence of secondary branching in the sporangiophores, and the coarsely verrucose stylospore wall suggest that these cultures should be referred to *M. vantieghemi* Bachmann, which Linneman (1941) recognized as a separate species though Zycha (1935) regarded it as synonymous with *M. polycephala*. These two characters vary greatly in this group of species which includes:

(i) *M. polycephala* with primary branching only in the sporangiophore and with finely echinulate stylospores.

(ii) *M. polycephala* var. *angusta* Linneman with verrucose stylospores.

(iii) *M. polycephala* var. *canina* (Dauphin) Linneman with simple or weakly branched sporangiophores and coarsely echinulate stylospores.

(iv) *M. vantieghemi* with secondary branching and verrucose stylospores.

(v) *M. vantieghemi* var. *cannabis* Dauphin and var. *raphani* (Dauphin) Linneman with secondary branching and finely echinulate stylospores.

Since these species are infrequently found and the published descriptions, though excellent, are usually based on a small number of isolates (often only one), it is difficult to assess the importance of the characters used to separate them. In particular, the degree of branching of the sporangiophores in a single isolate may vary with the medium on which it is grown. At present it seems more satisfactory to place them together under *M. polycephala* and to expand the description of this species to include the possibility of secondary and tertiary branching of the sporangiophores, and variation in the decoration of the stylospores. The closely related *M. reticulata* van Tieghem & le Monnier (1873), in which the degree of branching of the sporangiophores also varies, and *M. echinulata* Harz (1871) are separated from *M. polycephala* by the possession of larger, decorated (reticulate and echinulate) sporangiospores.

MORTIERELLA sp. Two cultures, M155 and M156, isolated from the *Salicornia* zone, cannot be reconciled completely with any species so far described. The substrate mycelium forms a lobed rosette of very variable appearance. The lobes may be pointed or rounded, large or small, and may vary in a single colony (Pl. 20, fig. 4). The aerial mycelium forms tufts or ridges over the lobes. Sporangiophores are sparsely formed on any medium and are borne singly on aerial hyphae, are 50–200μ long (mostly about 100μ) and taper from 2·5 to 5μ at the base to 1–2μ at the apex. Many are simple, but a single branch (rarely more) may arise about midway along the main axis and reach to about the same height (Text-fig. 3*e*). The sporangia are 7–15μ diam. (mostly 10–12μ) and usually contain 10–15 spores. The wall dissolves readily, leaving a small collar. The spores are smooth, round or slightly oval, and rather uniform in young cultures, 2–4μ diam., at least 60% measuring 2·5–3μ. In older cultures some larger, oval spores, about 3 × 5μ, may be mixed with the smaller round spores in the same sporangium (Text-fig. 3*f*). No chlamydospores or zygospores have been seen. Both cultures have the typical garlic-like smell of the genus.

These cultures might possibly be regarded as representing a small-spored variant of *M. minutissima*, which they resemble in general habit. They have some points in common with *M. parvispora* and with *M. gracilis* Linneman (1941), but the sporangia and sporangiophores are smaller than in either species. The sporangiophores of the former typically show abundant mixed branching from the upper part of the main axis, and while those of *M. gracilis* are slender with sparse cymose branching, the first branch arises from the lower part of the main axis, the branches are very long and the whole structure is usually long (up to 600 μ) and straggling. The sporangia and sporangiophores of M 155 and M 156 more closely resemble those of *M. turficola* Ling-Young (1930), and the slight difference in spore size (1·8–2 μ in *M. turficola*) could perhaps be due to the method of measurement used.

Ling-Young emphasized the close relationship between *M. turficola* and *M. pusilla* Oudemans & Koning (1902), which was originally described as having a lobed colony with woolly aerial mycelium, slender, simple, very slightly tapered sporangiophores 130–200 μ long, sporangia 20–24 μ diam. and round spores 3 μ diam.

It is unfortunate that Zycha (1935) classed as variants of *M. pusilla* certain species which have an entirely different growth habit. These species, which include *M. isabellina* Oudemans & Koning, form a close, velvety turf, sometimes with very narrow, regular zones, sparse aerial mycelium, and sporangiophores which arise always from the substrate mycelium. This growth habit was used by Linneman (1941) to separate a distinct group of species from the rest of the genus. All other species of *Mortierella* have woolly or cottony aerial mycelium, submerged mycelium which almost always forms lobes or broad, irregular zones (the 'rosette' habit) and sporangiophores which typically arise from aerial hyphae. Linneman followed Zycha in placing *M. pusilla* with the first group, and indeed named the group 'Sectio Pusilla'. Her new species *M. gracilis*, which has the rosette habit and which resembles *M. pusilla* except in having branched sporangiophores, was not placed in this group, nor was *M. turficola*, which Ling-Young described as resembling *M. pusilla* more closely than it resembled *M. isabellina* and other species described by Oudemans & Koning (the only species then known to possess small, round spores).

If the distinction made by these authors between the two mycelial habits (a distinction found by Linneman to be correlated with other differences) is accepted, it is clear that *M. pusilla*, as well as *M. gracilis* and *M. turficola*, should be excluded from the group which has the habit of *M. isabellina*. Their relationship to *M. minutissima* is less clear, but isolates M 155 and M 156 may suggest a link. It is proposed tentatively to refer these isolates to *M. turficola* Ling-Young, with the proviso that the relationships of this and certain other small-spored species of *Mortierella* require clarification. The description by Bayliss Elliott of a branching variety of *M. pusilla* from salt marsh mud is too brief to be of much help in this connexion.

DISCUSSION

In view of the general occurrence of *Mortierella* species in most soils the presence of the species described here is not surprising. Of the six species isolated from these alkaline muds the two most frequently represented (*M. alpina* and *M. minutissima*) were regarded by Linneman (1941) as typical of weakly acid to alkaline soils (pH 6–7·8). She considered that the pH value is one of the chief factors governing the distribution of *Mortierella* species in soil. She found *M. spinosa* in soils ranging from pH 5·6 to 7·8, and *M. parvispora*, found only once in this investigation, in soil at pH 5·2. It is interesting that Brown (1958) found *M. alpina* to be prevalent in alkaline sand-dune formations, while *M. parvispora* occurred only in acid dunes, as did *M. isabellina*, *M. marburgensis*, and *Mucor ramannianus* (*Mortierella ramanniana* (Moeller) Linnem. 1941), none of which have been found in the salt marsh muds. Brown, evaluating the growth of some of her isolates at different pH values, found that the growth of *M. alpina* was stimulated by increasing pH up to 8 (the limit of the experimental scale used), while the max. for *M. parvispora* was at pH 6, though it still grew well at pH 8. Little is known of the pH relationships of *M. polycephala* and *M. turficola*.

In common with other fungi of the salt marsh these species of *Mortierella* must be able to tolerate high salt concentrations, though not necessarily to grow in such concentrations. Preliminary studies have shown that several isolates of *M. alpina* can grow, though with decreased activity, in salt concentrations up to 4 %. This species has also been found by one of us (M. T.) in soil at the base of a limestone cliff, at high spring tide level, while the maritime dunes studied by Brown must also show fluctuating salt concentrations.

The sporadic occurrence of *M. parvispora*, *M. polycephala*, *M. spinosa* and *M. turficola* could be due to their being rare in the mud. It could also be due to their recent arrival on organic debris in fresh silt, in which case they could be regarded as 'salt marsh transients'. On the other hand, *M. alpina* and possibly *M. minutissima* must be regarded as 'salt marsh inhabitants' (cf. Garrett, 1956) because of their widespread occurrence.

The number of isolates of *M. minutissima* is not sufficient to indicate any difference in population levels in the area studied, but *M. alpina* shows a well marked and regular increase in its representation in the fungal flora, from approximately 2 % in the bare mud to 6 % in the *Salicornia–Spartina* zone and to 16 % in the mature *Puccinellia* sward. This landward increase could be due to the additional organic content of the mud with progressive colonization by higher plants, but other factors, such as aeration, may also be involved.

The writers wish to thank Prof. C. G. C. Chesters for helpful criticism of the manuscript, Mr T. R. G. Gray for providing the information on the physical conditions in the mud, and Mr D. J. Allen for taking the photographs.

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EXPLANATION OF PLATE 20

Cultures on malt agar showing various forms of the 'rosette' habit.

Figs. 1, 2. *Mortierella polycephala* (M 148).
Fig. 3. *M. minutissima* (M 149).
Fig. 4. *M. turficola* (M 155).

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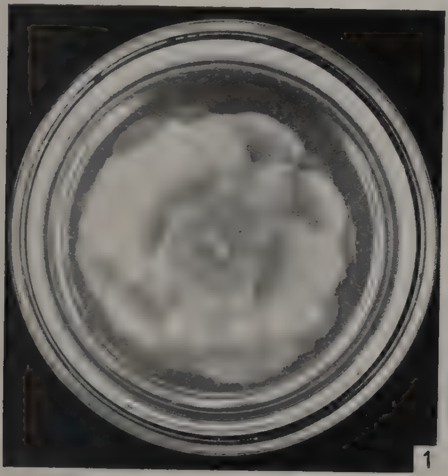

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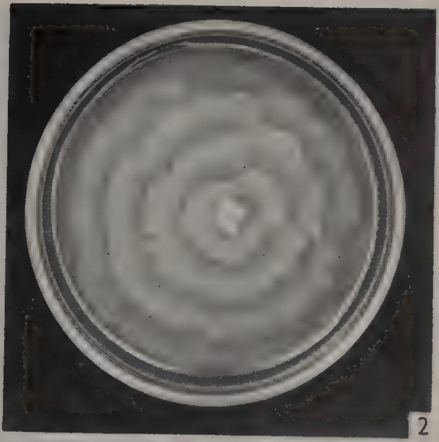

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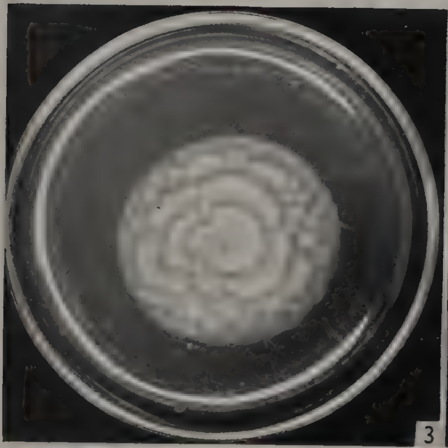

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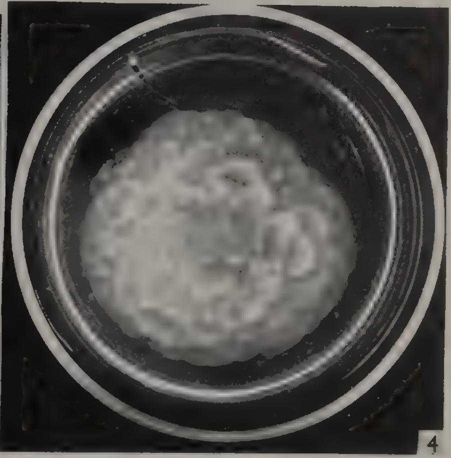

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Trans. Brit. mycol. Soc. **44** (2), 253–264 (1961).

STUDIES ON *MYCOSPHAERELLA BRASSICICOLA* (DUBY) OUDEM

By D. M. DRING*

Department of Botany, University of Exeter

(With 5 Text-figures)

The nomenclature, morphology, and life history of *Mycosphaerella brassicicola* are discussed, and it is concluded that the *Phyllosticta* stage is wholly spermatial in function.

Mycosphaerella brassicicola, in addition to its intrinsic interest, is the cause of a serious disease of cultivated brassicas in many parts of the world, including south-west England, but until now has not been the subject of detailed study in Great Britain. The object of this study is to elucidate the life cycle and other points which are the subject of conflicting references.

NOMENCLATURE OF THE FUNGUS

The nomenclature of both perfect and imperfect states is confused, and it is seldom that two authors are in agreement as to the names and citations. Weimer (1926) has, however, given a detailed account so that only additional information will be discussed at length here.

The earliest references are to the imperfect state of the fungus, the original description being that of Chevallier (1826) as *Asteroma brassicae*. McAlpine (1901) renamed the imperfect state *Phyllosticta brassicicola*. Saccardo (1884) described a similar fungus on swede as *P. napi*, and Grove (1935) considered that this may have been referable to *P. brassicicola*. Type material of Saccardo's fungus was examined during the present study, and, although it was impossible to be completely satisfied that it belongs to this species, no observation was made which precludes the possibility. *Phoma brassicae* Thüm. (Saccardo, 1884) and *P. oleracea* Saccardo (1884) are considered by Cunningham (1927) to refer to *P. lingam* (Fr.) Desm. This is also true of *Phyllosticta brassicae* Westendorp (1851) and *Depazea brassicae* Currey (1859), though these have sometimes been confused with *P. brassicicola*. Weimer's conclusion that the correct name for the imperfect state is *P. brassicicola* McAlp. must be accepted for the moment. It is noteworthy that, although the first epithet was '*brassicae*' in *Asteroma brassicae* Chev., this cannot now be used for this *Phyllosticta* as the combination would be a later homonym of Westendorp's binomial.

There would seem to be no doubt that the correct name for the perfect state is *Mycosphaerella brassicicola*. It is merely the author citations which are in doubt. The first known description of the perfect state is by Duby (1830) as *Sphaeria brassicaecola* Fr. ined. The description by Fries has never been found so that it seems necessary to accept Duby's account as the first description. The fungus was independently transferred in the same year to the genus *Mycosphaerella* Johanson (1884) by Oudemans (1897) and Lindau (1897) and the actual priority would be difficult to establish, as 'Engler & Prantl' bears no date other than '1897'. In this paper, however, preference has been given to Oudemans's transfer over that of Lindau, in conformity with the British Mycological Society's *List of Common British Plant Diseases* (1944), and in deference to the fact that Oudemans seems to have transferred more *Sphaerella* spp. to *Mycosphaerella* than did Lindau. Thus, the best author citation for *Mycosphaerella brassicicola* seems to be (Duby) Oudem. Some details of the synonymy of the two states are given in Table 1.

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Table 1. *The synonymy of* Mycosphaerella brassicicola

Perfect state

Mycosphaerella brassicicola (Duby) Oudemans (March 1897) [as '(Duby) Johanson']
    ≡ *Sphaeria brassicaecola* Duby (1830)
    ≡ *Sphaerella brassicaecola* Cesati & de Notaris (1863)
    ≡ *Sphaerella brassicicola* (Duby) Ces. & de Not. (Saccardo, 1882)
    ≡ *Mycosphaerella brassicicola* (Duby) Lindau (1897)
        = *Dothidea brassicae* Desmazières (1842)
        = *Sphaeria brassicae* Berkeley & Broome (1852),
            non Bolton,* *non* Klotzsch†

Imperfect state

Phyllosticta brassicicola McAlpine (Grove, 1935)
    ≡ *Asteroma brassicae* Chevallier (1826)
    ≡ *Phyllosticta brassicaecola* McAlpine (1901)
    ≡ *Phyllosticta brassicicola* Grove (1914)
        = ? *Phyllosticta napi* Saccardo (1884)

* *Sphaeria brassicae* Bolton (1789) = *Sclerotium semen* Tode (*fide* Fries, 1822), and is the sclerotial stage of a *Typhula* sp.

† *Sphaeria brassicae* Klotzsch is a *Sordaria* sp. *fide* Oudemans (1878). It could hardly conform to the modern definition of *Sordaria* but must be different from Berkeley & Broome's fungus.

SYMPTOMS OF THE DISEASE

Those parts of this investigation which deal with the disease, its symptoms, epidemiology and control are discussed elsewhere (Dring, 1962) but it is appropriate to give some details here.

By far the most important hosts of the fungus are cultivars of *Brassica oleracea* L., particularly Brussels sprout and broccoli. The fungus is apparently incapable of attacking plants not included in the genus *Brassica* L. Practically all parts of the plant above ground may be attacked, but the most typical symptom is a ringspot which is produced most commonly on the laminae of the outer leaves. Ringspots start as small brown necrotic specks which enlarge into roughly circular lesions with brown background and containing black fruit-bodies in typically zonate arrangement. On the living leaf the lesion is bounded by a narrow, water-soaked area, and a zone of chlorotic tissue surrounds the whole. Development of the fruit-bodies is hypophyllous at first, amphigenous later. Fruit-bodies on leaves still attached to the plant almost always belong to the *Phyllosticta* state, and there has developed, at any rate in Britain, an idea that the perfect state is rather rare (Grove, 1935). Inspection of fallen leaves, however, usually reveals large numbers of perithecia.

MORPHOLOGY AND LIFE HISTORY OF THE FUNGUS

The ascospores (Fig. 1 *a*) are colourless, thin-walled, bicellular, and usually slightly curved. The two cells are almost equal in size but one is slightly broader than the other. Typically, one large, and one to three smaller oil droplets are conspicuous in each cell. There is only a slight constriction at the septum immediately after discharge, but since, in a moist atmosphere or on contact with water, the ascospores quickly begin to swell, the constriction is often more noticeable. For the same reason it is difficult to measure ascospore size accurately. On discharge they are 18–20–23 × 3·5–4 μ but they quickly increase in size, and spores from the same sample (from Brussels sprout leaves, Exeter, February, 1955) as those of which the sizes are recorded above measured 18–22–25 × 3·5–4–5 μ some 30 min.

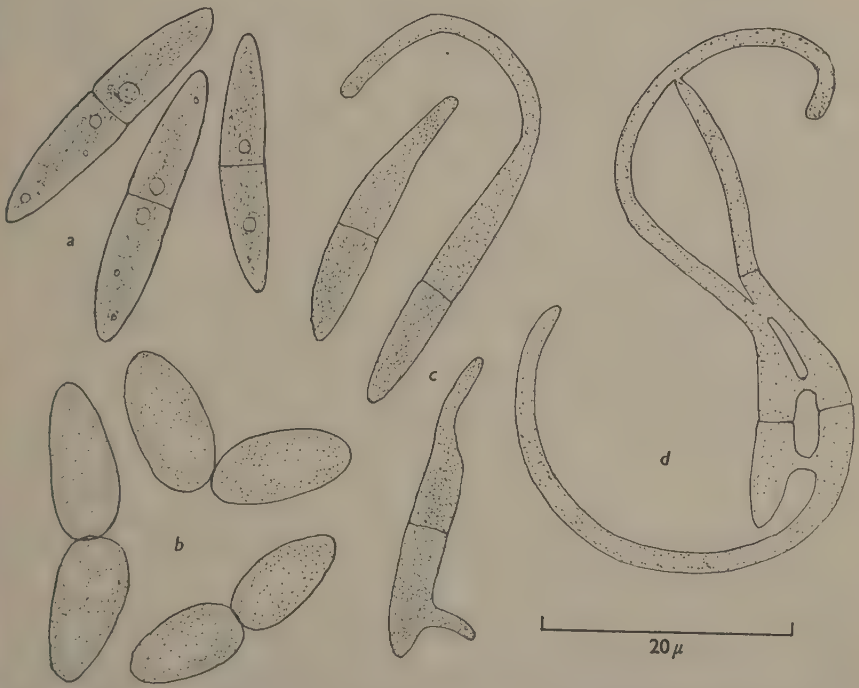

Fig. 1. Ascospores of *Mycosphaerella brassicicola*: *a*, recently discharged spores, showing oil droplets; *b*, ascospores swollen with imbibed water; *c*, germinated ascospores, showing that either or both cells may germinate, terminally or laterally; *d*, pseudomyxis of germinated ascospores.

later. This phenomenon may partly explain discrepancies in ascospore sizes recorded in the literature.

Experiments showed that germination decreased from 99 % at 100 % R.H. to nil at 90 % R.H. The first sign of germination is an increase in spore size, the constriction at the septum becoming more marked. Sometimes the two cells become partly separated (Fig. 1 *b*). The protoplasm streams actively, and the oil droplets disintegrate, finally becoming submicroscopic. There is no germ pore, and either one or both cells may germinate, usually from the apex, but often laterally (Fig. 1 *c*). Two types of germ tubes were noted, and are here called primary and secondary. Primary germ-tubes, 2–3 μ diam., develop into hyphae. Sometimes more than one may be produced from one cell of the ascospore. Secondary germ-tubes are produced less frequently, usually by spores which have failed to germinate in any other way. They are approximately 1·5 μ diam., and through them the spore takes part in what is apparently pseudomyxis, either with another spore or with a hypha (Fig. 1 *d*). No satisfactory method was found to stain the nuclei, so that it is not known if there is any transfer of nuclear material through these tubes. In view of the strong tendency to anastomosis exhibited by the mycelium of the fungus, pseudomyxis of the ascospores is perhaps not very remarkable.

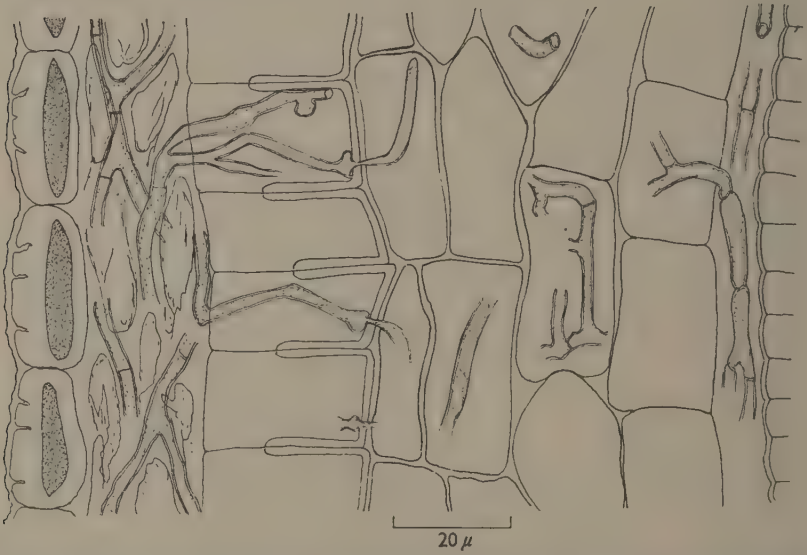

Fig. 2. Penetration of cabbage testa by mycelium of *M. brassicicola*, showing penetration pegs in the prismatic layer of the testa. The epidermis of the testa is to the left. The layer of cells on the extreme right is the epidermis of the cotyledon.

A primary germ-tube enters a leaf of *Brassica oleracea* by penetration of a stoma. Several hyphae were observed entering the host by this means (Fig. 3 *a*), though none was seen to penetrate the cuticle. This is in contrast to penetration of the prismatic layer of the testa which is by means of penetration pegs produced by mycelium in the subepidermal layer (Fig. 2), and to penetration of young pea cuticles by germ-tubes of *M. pinodes* (Berk. & Blox.) Stone (Baumann, 1953).

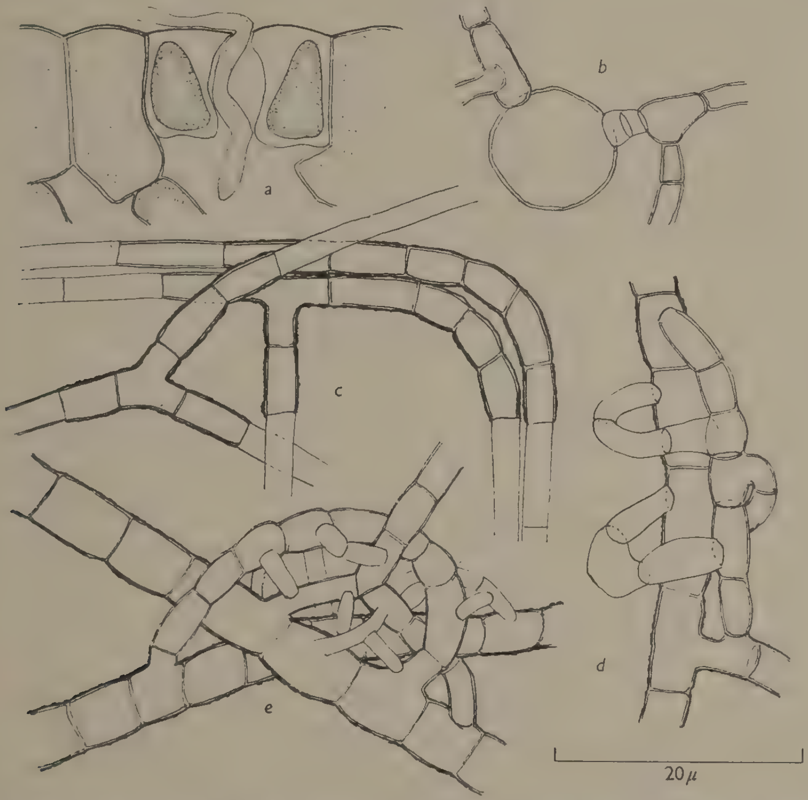

Fig. 3. Mycelial characters of *M. brassicicola*: *a*, hypha entering stoma of cabbage lamina; *b*, inflated hyphal body resembling a chlamydospore; *c*, hyphal wall thickening and melanin deposition, showing sites at which this typically occurs. The centre of the colony is toward the left; *d*, anastomosis of thick-walled mycelium, showing semicircular hyphae; *e*, fruit-body primordium developing from such a system as is figured in *d*.

On entering the substomatal chamber the hyphae, at this stage hyaline and averaging 2·3 μ diam., begin to spread between the host cells. Hyphae were never observed to penetrate living cells. A toxin is produced (Dring, 1962) causing the host cells to collapse and die, whereupon they are frequently penetrated by hyphal branches.

As the mycelium becomes older, the diameter of the hyphae increases up to a maximum of about 7 μ and constrictions appear at the septa, the occasional result of which is to produce a subspherical body up to 15 μ diam. resembling a chlamydospore (Fig. 3*b*). None of these bodies has been observed to germinate, but similarly aged and morphologically more typical cells are certainly capable of renewed growth. The normal mycelium becomes, with age, encrusted with dark brown melanin deposits, giving the wall a verrucose appearance. Melanin is probably also liberated into the host tissues, since the lesions are darker than similar tissue killed by other means. Noticeable quantities of the pigment are liberated into certain media when the fungus is grown in culture.

There is a marked tendency for the mycelium to produce the melanin deposits unevenly along the lengths of the hyphae (Fig. 3*c*). These deposits usually form at points where branching occurs and this probably indicates that these junctions are centres of greater metabolic activity, where translocation streams converge and nutrients are easily available. Fruit bodies begin to develop at these points. The primary hyphae involved in the junction produce secondary branches which grow in a semi-circle, eventually meeting the parent hyphae again (Fig. 3*d*). The secondary hyphae themselves produce laterals which make connexion with the original parent hyphae or with any of their subsequent branches (Fig. 3*e*). By this system of anastomoses a knot of mycelium is built up, finally becoming a pseudoparenchymatous fruit-body primordium (Fig. 4*a*).

Some 3–4 weeks after infection, necks of the so-called pycnidia begin to project through stomata and other orifices in the dead portion of the lesion. The 'pycnidium' is a flask-shaped body, 39–63–90 μ diam., consisting, immediately before maturity, of a tightly packed mass of thin-walled, hyaline pseudoparenchyma surrounded by a peridium two to three cells in thickness made up of thick-walled, dark-coloured cells (Fig. 4*b*). The neck is composed of similar cells elongated along the axis of the fruit-body. 'Pycnidia' are occasionally produced internally, e.g. on the inner wall of the mature carpel or inside a very decayed leaf. In culture, though most are produced on the surface of the mycelium, a fair number are embedded within it, tending to be atypical in structure.

The PAS staining method (Dring, 1955) revealed that the 'pycnospores' are formed inside the thin-walled cells of the fruit-body by division of the protoplast into four, possibly sometimes into a smaller number. The resulting daughter protoplasts are extruded through a sterigma, of which there is usually one (Fig. 4*c*) but occasionally two (Fig. 4*d*) per mother cell. They apparently remain attached to their sterigmata until pushed from them by the next emerging single 'spore', or until the empty mother cell collapses. This method of production and discharge has been described for the spermatia of *M. personata* Higgins (1929) and *M. tulipferae* (Sch.) Higgins (1936), and many other *Mycosphaerella* species.

A frequent result of one 'spore' being pushed from the sterigma by the emergence of the next is that the two become stuck together, end-to-end, to give an apparently bicellular structure. Strictly, however, the 'pycnospores' are unicellular, densely protoplasmic, cylindrical bodies, 0·75–1 × 3–4 μ. They are discharged into the developing cavity of the fruit-body together with a quantity of mucus presumably derived from exhausted and aborted mother cells. In a well matured fruit-body, 'pycnospores' are commonly formed at the apices of columns of maturing mother cells. At maturity of the fruit-body the mucus absorbs water, and the contents of the cavity begin to extrude through the ostiole, accumulating in a globule.

Although Cooke (1906) and Westendorp (1851) have described *M.*

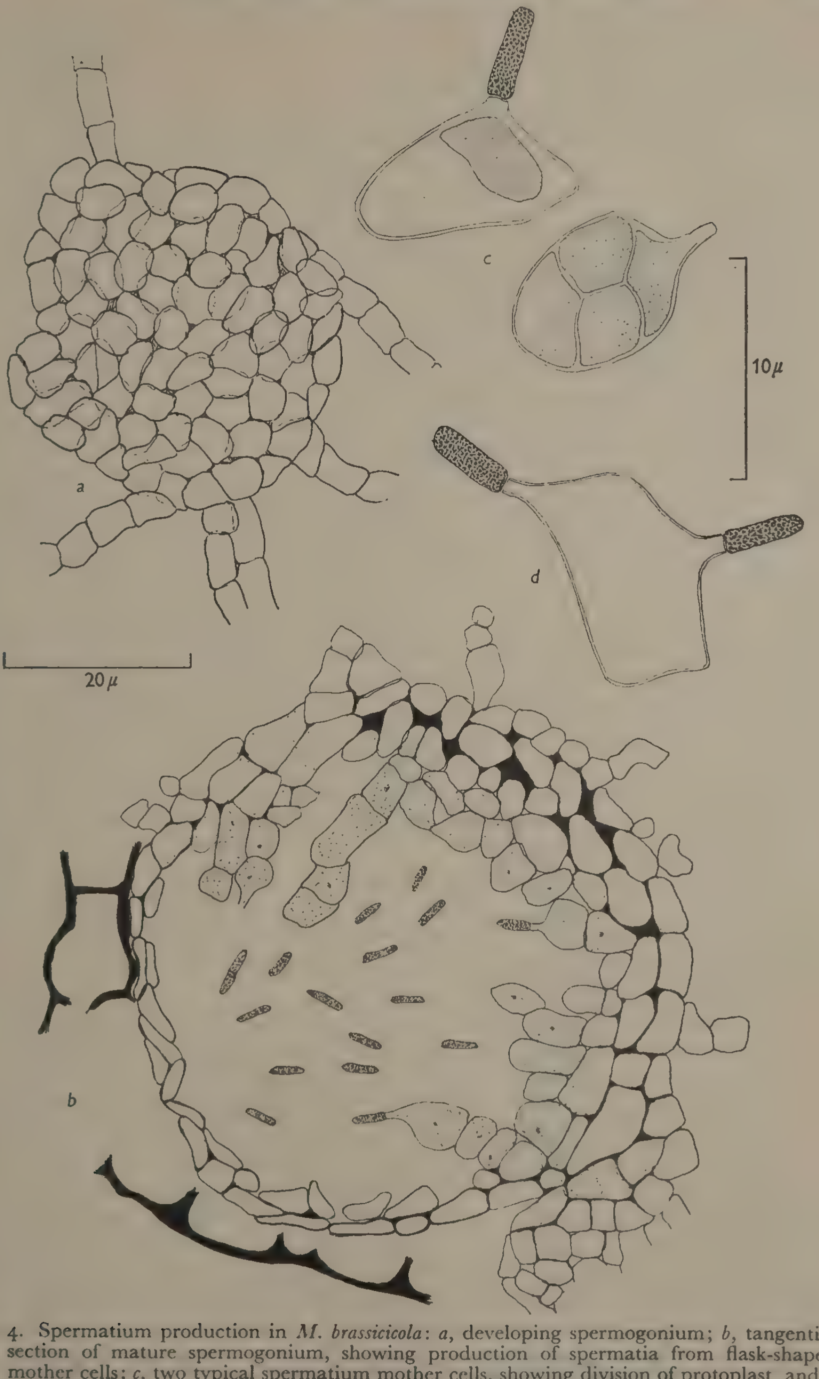

Fig. 4. Spermatium production in *M. brassicicola*: *a*, developing spermogonium; *b*, tangential section of mature spermogonium, showing production of spermatia from flask-shaped mother cells; *c*, two typical spermatium mother cells, showing division of protoplast, and a spermatium borne on a sterigma; *d*, atypical mother cell with two sterigmata.

brassicicola as producing tendrils of pink pycnospores, it is probable that they were mistakenly describing the spore production of *Phoma lingam*, which forms lesions on *Brassica* laminae very similar to those of *M. brassicicola*.

The fruit-body collapses when it has discharged its contents. It does not apparently develop into a perithecium. Disintegrating remains of exhausted 'pycnidia' have often been observed and it is doubtful if these are capable of regeneration.

'Pycnospores' have never been observed to germinate. Snyder (1946) tried various methods in fruitless attempts to induce germination. These methods included placing mixtures of 'pycnospores' and ascospores on various agar media and in drops of water, with and without cabbage leaf tissue added, and incubating at laboratory temperature and out-of-doors. In all cases ascospores had germinated after 24 hr., but no 'pycnospore' was ever observed to do so. Twenty leaves were inoculated with ascospore and twenty with 'pycnospore' suspensions. Ten of each were placed outside (in the Californian winter), and the remainder incubated in the laboratory. Infection occurred only on the leaves inoculated with ascospores and incubated out-of-doors. Weimer (1926) kept mounts of sectioned 'pycnidia' for 3–4 days, but in no case was germination observed.

Each of the above methods, with minor alterations, was attempted not less than three times during the present study. In addition, a wider range of temperatures was used. No germination of 'pycnospores' was seen. Further, thousands of observations were made on 'pycnospores' in nature but none was ever seen to be in process of germination. Snyder (1946) mentions that no 'tear-stain' secondary infection occurs in the field and concludes that this is further evidence that the 'pycnospores' are unable to germinate. This observation and conclusion is strongly supported by the results of the present study.

Perithecia are usually seen on older leaves which have become yellow and fallen from the plant, though they are sometimes found on sound leaves. They are apparently initiated in the same way as 'pycnidia' but develop into larger, more nearly spherical bodies (Fig. 5*a*), 70–87–124 μ diam.

The internal construction of the protoperithecium is similar to that of the immature 'pycnidium', the two being virtually indistinguishable. In the pseudoparenchyma at the base of the protoperithecium, a number of cells become differentiated into ascogonia. None of these has ever been seen until after fertilization, when they appear as cylindrical structures, 10–20 × 5–8 μ, with a lateral or subterminal trichogyne (Fig. 5*a*). The apparently lateral insertion of the trichogyne at this stage may be due to unequal growth of the fertilized ascogonium. The trichogynes are narrow, unbranched, hyaline hyphae, 1 μ diam., which protrude from the neck of the fruit-body to a length of up to about 50 μ. As many as ten to fifteen trichogynes can frequently be seen projecting from one fruit-body, indicating a similar number of ascogonia. 'Pycnospores' are often seen adhering to these trichogynes (Fig. 5*b*) though cytological evidence of their fusion was not obtained.

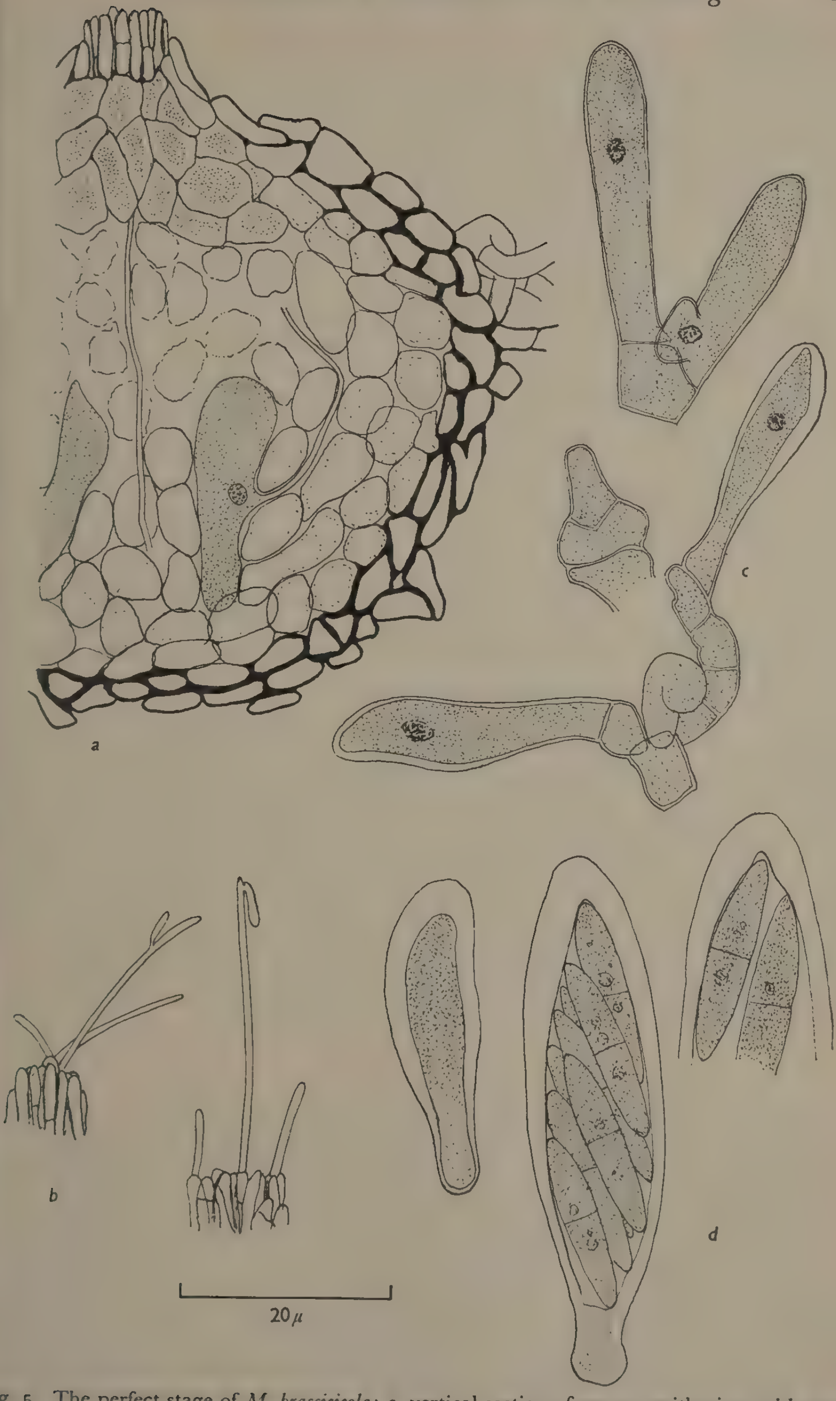

Fig. 5. The perfect stage of *M. brassicicola*: *a*, vertical section of young perithecium with ascogonia and trichogynes; *b*, spermatia in contact with projecting trichogynes; *c*, ascogenous hyphae, showing branching and cell division stages; *d*, bitunicate asci in various stages of maturity.

Although the ascogonia are not more chromophilic than the surrounding cells, the ascogenous hyphae stain deeply in gentian violet and are hence easily studied. They are 5–8 μ diam. and show typical crozier formation (Fig. 5*c*). They frequently branch several times near their origin and each of the resulting branches typically develops basipetally into a number of asci (Fig. 5*c*).

Since each perithecium may contain several ascogonia and each ascogonium produces a branching ascogenous hypha, every branch of which bears a succession of asci, the number of asci produced in each perithecium is substantial, and due to succession in maturity of the asci the perithecium goes on producing ascospores over a period of several days. Ripe asci measure approximately 15 × 40 μ, each containing eight spores (Fig. 5*d*). The ascus is bitunicate from its inception, and at maturity the outer wall is inelastic whereas the inner is capable of being stretched to many times its original size.

When the correct conditions for discharge are present the ascus absorbs water, causing the outer wall to rupture, usually by a slit at its apex. The inner ascus, relieved of the restraint of the outer wall, quickly elongates until its tip is pushed out of the ostiole for a distance of about 80 μ when the perithecium is immersed in water, but never so far when in air or in more nearly isotonic solutions. The spores are then ejected in succession through a pore in the tip of the ascus wall. The spores are arranged with their broader ends towards the apex of the ascus, resulting in more forceful discharge (Ingold, 1954). Also, as a consequence of the orientation of the spore, the relatively wide forward end takes longer to pass through the pore than does the narrower end. This results in a definite interval, often only just perceptible but sometimes of about ¼ sec., between discharge of one spore and the next. This prevents the spores from becoming stuck together at discharge and results in more efficient distribution. Experiments showed that the largest proportion (34 %) of spores was discharged to a distance of 4–5 mm.; a few (1 %) reached 7–8 mm. Experiments with glucose solutions of various osmotic pressures showed that discharge occurred in solutions of up to 17·5 atm. osmotic pressure. This would not, however, occur in nature, being unnecessary and even mechanically impossible because of the delicacy of the ascus wall.

Discussion

The life cycle consists of a perfect state, usually though not always found on dead or dying leaves of the host, alternating with a single imperfect state on living leaves. No trace has been found of a state with pink spores produced in tendrils.

Snyder (1946) suggests that the *Phyllosticta* state is not pycnidial but spermatial in function, a conclusion which is based on the small size of the 'pycnospore' and its apparent inability to germinate. This view is supported by results obtained during the present study. The discovery of ascogonia with trichogynes suggests that spermatia may be present in the life history of the fungus. The fact that 'pycnospores' are produced endogenously in a flask-shaped mother cell in just the same way as the undoubted spermatia of other *Mycosphaerella* spp., that they were frequently found in contact with trichogynes, and could not, in spite of repeated attempts, be induced to germinate, leaves little doubt as to their spermatial nature.

However, from a survey of the literature the impression is gained that the perfect state is not sufficiently frequent in the British Isles to account for the commonness and, at times, disastrous consequences of the disease. Grove (1935) states that it is the *Phyllosticta* state which is responsible for spreading the disease and that ascospores serve simply to carry the fungus over the 'dead period'.* Such statements spring from the belief that, as in many fungi of this type, the perfect state is decidedly rare, being found chiefly on overwintered leaves of the host. In Devon, throughout the period October 1953–October 1956, ascospores were always to be found (with the exception of periods of little over a month in midsummer). Large numbers of ascospores were always found about 4 weeks before the occurrence of serious outbreaks and infection was reduced almost to nil at times when ascospores were very rare. It is maintained, therefore, that there were always sufficient ascospores present to account for all infections, and hence there is no necessity to assume the presence of any imperfect spore to explain the incidence of the disease.

Comparison of British material with specimens, cultures and literature from other countries where the disease is common suggests that there is no significant departure in any part of the world from the biology and life-history as described above.

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* It is uncertain what is meant by 'dead period' in this context. Brassica growing is conducted in England at all times of the year.

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FRUIT-SPOT ('SPECKLE') OF JAMAICAN BANANAS CAUSED BY *DEIGHTONIELLA TORULOSA* (SYD.) ELLIS

II. FACTORS AFFECTING SPORE GERMINATION AND INFECTION

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(With Plate 21 and 3 Text-figures)

Some factors affecting germination of conidia of *Deightoniella torulosa* have been studied. In tap water on glass or on the surface of banana fruits, approximately 50% germination occurs after 1 hr. incubation at 28–30° C. After 4–6 hr., nearly 90% of conidia have germinated. Germination does not occur below 95% R.H.: percentage germination in a water film is markedly higher than that at 100% R.H. Using a series of citrate-phosphate buffers, the optimum pH for germination and germ-tube elongation is in the range 4–6. Substances present in water extracts of banana leaves and fruit peel stimulate germination, as do drops of distilled water that have remained on the surface of young banana fruits for 24 hr. Solutions of several compounds present in synthetic nutrient media provide better germination than does distilled water; some carbohydrates tested are particularly favourable.

The longevity of *D. torulosa* conidia is dependent on external humidity. Viability is lost within 3–4 days at R.H. $< 95\%$. At 98% R.H., a small number of conidia may be viable after 10 days storage.

The severity of fruit-spot caused by *D. torulosa* increases progressively with advancing maturity of fruit. Spotting tends to be most intense near the tips and on the inner sides of individual fingers. Presence of dew or rain water appears to be essential for successful infection by *D. torulosa*. Experimental inoculations have shown that resistance to infection increases to some extent as the fruit matures. The severity of fruit-spot undergoes seasonal variation, being highest after the rainy periods during April–May and September–October.

D. torulosa sporulates on diseased, dead or dying leaves, withering flower parts and on transition leaves and bracts. The relative importance of these inoculum sources in relation to fruit-spot epidemiology is discussed. Removal of dead or dying 'trash' leaves from the plantation at frequent intervals is recommended.

Isolates of *D. torulosa* from fruit-spots are able to cause a spotting of banana leaves ('black-spot') and a rotting of the tips of banana fruits ('black-tip'). Conversely, isolates from 'black-spot' and 'black-tip' lesions may cause fruit-spot. A black scab-like fruit-spot, also caused by *D. torulosa*, occurs in a few widely separated plantations.

In previous communications (Meredith, 1960, 1961), it was reported that a spotting ('speckle', 'swamp-spot') of Jamaican 'Lacatan' banana fruits is caused by *Deightoniella torulosa* (Syd.) Ellis. After germination has occurred on the fruit surface, appressoria are produced and an infection hypha penetrates the apparently uninjured cuticle and epidermal wall.

The present paper is an account of further studies on infection by *D. torulosa*, with particular reference to some factors affecting the process.

FACTORS AFFECTING SPORE GERMINATION

Germination of a spore after it has come to rest on the surface of the host is an essential part of the life cycle of a pathogenic fungus. In this investigation, conidia were obtained from 7- to 10-day-old colonies of *D. torulosa* growing on potato-dextrose agar. Dry films of conidia were prepared by placing a drop of a conidial suspension on the test surface, for instance glass, banana leaf or fruit, and drying quickly with the help of an electric fan.

Rate of germination

Percentage germination of conidia of *D. torulosa*, contained in tap-water films on a glass surface, was recorded at intervals; germ-tube length was also measured. In Table 1, data for percentage germination are means of three independent counts, each of not less than 200 conidia. Germ-tube measurements are based on thirty random observations.

Table 1. *Rate of germination of* Deightoniella torulosa *at* 28° C. *in water on a glass surface*

Time interval (hr.)	Mean % germination	Germ-tube length (μ) Mean	Germ-tube length (μ) Maximum	Appressoria
1	52·7	13·1	21·4	No
2	75·3	22·8	56·0	No
3	77·4	32·4	66·5	No
4½	86·8	47·7	94·5	Yes
6½	88·2	86·0	137·5	Yes

Observations made after 6½ hr. showed that there was little further increase in percentage germination (92 % at 12 hr.). In other experiments, it was found that rate of germination in tap water on the surface of banana fruits and leaves was comparable with that on glass. There was, however, a tendency for germ-tubes to be shorter and for appressoria to be developed sooner than on glass.

Conidia of *D. torulosa* produce one or several germ-tubes (Ashby, 1913). In the present experiments, germination up to 3 hr. was predominantly unipolar, a germ-tube emerging from the distal end only. After 4½ hr., many conidia produced another germ-tube at the conidiophore end. In a small number of cases a germ-tube was produced from one or more intermediate 'cells' of the conidium (Pl. 21, fig. 1). This appears to be an erratic feature of germination, for some isolates produced only terminal germ-tubes in tap water. Germination from intermediate 'cells' was never observed on the surface of naturally or experimentally infected fruit; it was either uni- or bipolar. Germination was equally good in bright sunlight, diffuse daylight or darkness.

Humidity

The effect of relative humidity on germination was studied. Dry films of conidia on glass and on the surface of banana-fruit peel were maintained under constant humidity conditions above saturated solutions of various salts selected from the list given by Lange (1952). Other conidia from the same culture were set up in films of tap water. The results obtained after 20 hr. incubation at 25 ± 2° C. are given in Table 2; germination percentages are means of three independent counts, each of at least 150 spores.

Percentage germination in a water film was considerably higher than that in an atmosphere of 100 % R.H. or less. In a saturated atmosphere, some germination may have been due to water condensing on the spore-bearing surface. Although there was some germination at 98 % R.H., germ-tubes did not exceed 10 μ in length nor were appressoria developed. In a water film, germ-tubes were up to 200 μ long and there was prolific appressorium development. There was a tendency for germination on the surface of fruit to be better than that on glass.

Table 2. *Relation of humidity to percentage germination of* Deightoniella torulosa *conidia*

	In water film	In moist air, % relative humidity					
		100	98	95	92	88	84
On glass	82·5	35·4	7·7	0	0	0	0
On banana fruit	97·0	47·6	14·1	0	0	0	0

Table 3. *Effect of hydrogen-ion concentration on germination of* Deightoniella torulosa *conidia*

pH	Mean % germination	Germ-tube length (μ)		Appressoria
		Mean	Maximum	
3	11·4	6·4	10·5	No
4	98·5	202·9	255·0	Yes
5	98·3	147·9	204·0	Yes
6	92·0	165·0	272·0	Yes
7	62·3	34·5	70·0	Yes
8	25·6	36·9	105·0	No
Tap water (pH 7·4)	83·2	84·0	157·5	Yes
Distilled water (pH 6·4)	58·0	39·9	91·0	Yes

Hydrogen-ion concentration of germination medium

According to Hawker (1950), spores of most species of fungi germinate best in acid media. The pH of the 'infection drop' in nature may be a factor of considerable importance in relation to infection by *D. torulosa*. Spores were germinated on the surface of banana leaves in drops of citrate-phosphate buffer solutions with initial pH values ranging from 3 to 8. Observations on percentage germination and germ-tube growth were made after 8 hr. incubation at 28–30° C. (Table 3).

Under these conditions, the optimum pH for germination and germ-tube growth was clearly in the range pH 4–6. It is of interest to note that tap water was more favourable than was distilled water, even though the former was slightly more alkaline. Possibly, impurities in tap water act as nutrients which stimulate germination. Another feature of interest was that at pH 4–6 germination was predominantly bipolar: in the other media tested, germination was chiefly unipolar, i.e. from the distal end of the conidium only.

Nature of germination medium

Substances of plant origin. Brown (1922) showed that if drops of water are placed on the surface of leaves or petals of various plants, substances diffuse into the water and stimulate germination of *Botrytis cinerea*. The possibility that similar exosmosis occurs from the surface of banana fruit was investigated. Immature bananas, collected approximately 10 days after opening, were washed thoroughly with distilled water and allowed to dry. Drops of distilled water were pipetted on to the surface and were allowed to remain there for 24 hr. The drops were then transferred to clean glass slides and conidia of *D. torulosa* were added. Extracts of banana leaves and fruit peel were prepared by adding 10 g. chopped tissue to 100 ml. distilled water. After soaking for 24 hr. the extracts were filtered and the pH of the filtrates determined. Drops of filtrate, to which conidia of *D. torulosa* had been added, were set up on glass slides.

Table 4. *Germination of* Deightoniella torulosa *in various media*

Medium	pH	Mean % germination	Type of germination	Appressoria
Distilled water	6·7	55·3	Chiefly unipolar (distal end only)	Few
Distilled water on fruit surface	—	89·2	Chiefly bipolar	Many
Distilled water extract of banana leaf	5·6	99·2	Chiefly bipolar	Many
Distilled water extract of banana fruit peel	6·3	97·2	Chiefly bipolar	Many

The percentage germination in these various media, and in distilled water only, was recorded after 16 hr. incubation at 28–30° C. The results are given in Table 4; percentages are means of five counts each of not less than 150 spores.

Germination in leaf- or fruit-peel extracts was better than in distilled water. The relatively high percentage germination in drops that had been on the surface of banana fruit suggests that substances diffused from the peel. No attempt was made to determine the chemical composition of the diffusate. It may be noted here that, in all of the experiments conducted so far, the best medium for germination was also that which supported the most vigorous germ-tube growth. In the present experiment, the mean germ-tube length in distilled water was 44·1 μ. Extensive branching and curvature of germ-tubes in the other media made it impossible to measure lengths accurately, but the mean was always considerably greater than that in distilled water. In extreme cases, a length of more than 500 μ was attained.

Temperature

Without thermostatically controlled incubators, it was not possible to investigate the effects of temperature on germination of *D. torulosa*. However, it may be noted here that high percentage germination was always realized at temperatures prevailing in the plantations on the plains of St Catherine (70–80° F.). It is of importance to know whether germination and infection can occur at temperatures inside the holds of refrigerated banana ships.

Longevity of conidia

The longevity of conidia of *D. torulosa* was tested under various humidity conditions. Dry films of conidia were prepared on clean glass slides, which were then stored inside desiccators containing different saturated salt solutions. Other slides were stored inside desiccators containing anhydrous calcium chloride, while yet others were maintained under laboratory conditions (R.H. 67–72 %). The initial viability of the conidia was checked by carrying out a germination test in tap water. Slides were removed at intervals and percentage germination in tap water was recorded after 12–16 hr. incubation at 28–30° C. (Table 5).

Table 5. *Longevity of* Deightoniella torulosa *conidia under different humidity conditions: percentage germination*

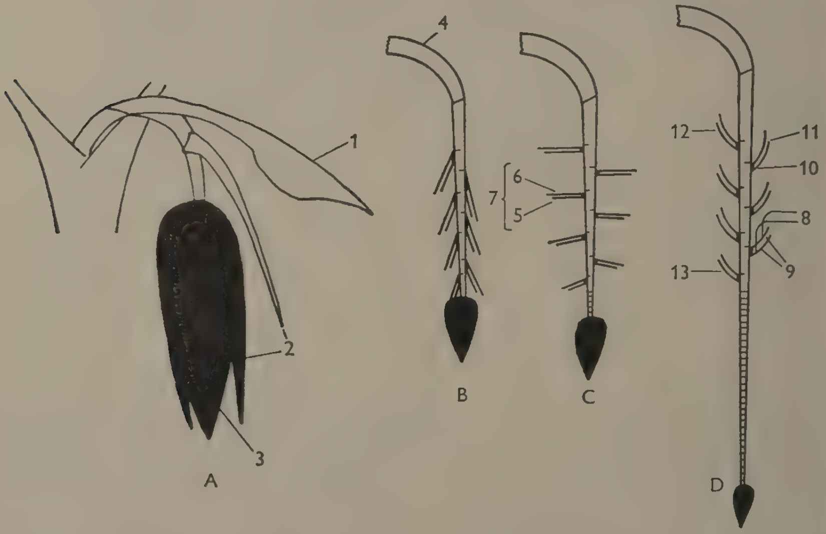
Storage interval (days)	Dry (over $CaCl_2$)	Laboratory conditions	At controlled humidities (% R.H.)			
			68·6	84·0	95·0	98·0
1	7·9	10·4	6·4	28·0	22·2	66·9
2	4·2	2·7	1·6	4·1	17·4	52·7
4	0	0	0	0	2·3	43·4
6	0	0	0	0	0	35·7
10	0	0	0	0	0	12·2
20	0	0	0	0	0	0

Controls { Without previous drying 76·5
After momentary drying 75·9

The results demonstrate that longevity is dependent on the external humidity. Below 95 % R.H., viability declined rapidly and was lost completely after 3–4 days. Decrease in viability was slower at 98 % R.H. The results for control conidia suggest that momentary drying had no significant effect on viability. From this experiment, it seems unlikely that liberated conidia of *D. torulosa* will, under plantation conditions, maintain their viability for lengthy periods. During hot, dry days, many of them will lose viability. During the night, those which are still viable and situated on banana leaves or fruits may be stimulated to germinate by contact with condensed dew. Similar depletion of the inoculum reserve probably occurs in rainy weather.

TERMINOLOGY RELATING TO BANANA FRUIT

Since the development of a bunch of banana fruits presents certain peculiar features, it is desirable to explain some of the terms employed throughout this paper. The young inflorescence ascends in the centre of the pseudostem and, when first visible at the top, is said to 'peep'. After complete emergence, the 'main-stalk' undergoes curvature through almost 180° so that the tip of the unopened inflorescence points nearly vertically downwards (Text-fig. 1A). The bunch has now 'shot'. It is 5–7 days after peeping before the spathe-like bract covering the large proximal 'hand' of 'fingers' (individual banana fruits) curls backwards and abscisses; this hand has now 'opened'. The more distal hands are exposed successively at intervals of approximately 1 day. When each hand first opens, the closely packed fingers point downwards towards the distal end of the main stalk and are pressed against the main stalk (Text-fig. 1 B). They then rise and, 15–20 days from peeping, project almost at right angles from the main stalk (Text-fig. 1 C). The uppermost faces of each finger are referred to here as the 'inner' faces and the lowermost as the 'outer' faces. Eventually the negatively geotropic reaction causes the fingers to assume an almost vertical position with the finger-tips pointing upwards (Text-fig. 1 D). The short stalk by which the finger is attached to the 'cushion' on the main stalk is known as the 'finger-stalk'.



Text-fig. 1. Diagrams illustrating the development of a bunch of bananas. A, a 'shot' inflorescence bud in which the hands are still completely covered by bracts; B, a 'shot' bunch in which the bracts have fallen away, thus exposing the uppermost hands (female portion of inflorescence); C, a 15- to 20-day-old bunch with fingers almost at right angles to main stalk, due to a negatively geotropic reaction; D, showing orientation of fingers at the time of harvest (80–90 days after 'shooting'). 1, 'spade leaf'; 2, bract; 3, unopened inflorescence bud; 4, main-stalk; 5, outer whorl of fingers; 6, inner whorl of fingers; 7, complete hand; 8, inner sides of fingers; 9, outer sides of fingers; 10, finger-stalk; 11, finger-tip; 12, proximal hand; 13, distal hand.

'Age' of fruit is expressed here as the approximate number of days from the time of opening. This is easily reckoned by counting the number of hands and scars on the main stalk distal to the hand in question. The total so obtained is a rough guide to the age. Thus '0 days' implies that the hand had opened on the day of examination. Occasionally it was necessary to use fruit that had not yet opened. In these cases a minus sign indicates that the bract was removed artificially some days before it would normally have fallen away. All observations reported in this paper were carried out on the 'Lacatan' variety of banana.

SOME FACTORS INFLUENCING INFECTION

Humidity

Earlier tests showed that conidia of *D. torulosa* fail to germinate at humidities lower than 98%. Unless the humidity at the surface of the fruit exceeds this value, it seems unlikely that infection will occur. This was tested in a further experiment. Dry spore films were set up on the peel of detached, 10-day-old fingers which were maintained at constant humidities inside small desiccators containing various saturated salt solutions or water. On other fruits, spores were placed on the peel in tap-water droplets. The numbers of spots developed after 5 days incubation at 28–30° C. were recorded. There was a high incidence of spotting on fruit with a water film, and on that maintained at 100% R.H. A few isolated spots developed at 98% R.H., but no infection occurred below 95% R.H. There is strong evidence that the majority of infections in the field occur while the fruit surface is covered with a film of water. It has been observed frequently that severe outbreaks of fruit-spot follow periods of heavy rainfall. One series of observations was particularly striking. A plot of bananas had been kept under constant observation from the time when the first bunches had 'shot'. Up till the beginning of the first week of June 1960 there was little rain and the incidence of spotting was correspondingly low. The plot was revisited after 7 days of almost continuous rainfall (17·9 in.). The contrast was remarkable, for the intensity of spotting on most bunches examined had increased to a level higher than that in any other instance previously observed. On 15- to 20-day-old bunches, spotting was restricted to the horizontally orientated inner sides of the fingers. This may be related to the tendency for conidia to impact chiefly on these sides and to the fact that water droplets are retained there for some time. On older bunches, water collects on the inner sides towards the finger-stalk, thus helping to explain the more intense spotting commonly observed in this region. Heavy finger-tip spotting was also apparent.

Maturity of fruit

Natural infection. The data recorded in Table 6 summarize the results of a field survey in which the relation of severity of fruit-spot to age of fruit was studied. The observations were made in May 1960, in plantations situated on the plains in St Catherine. In recording, a detailed examination of the large proximal hand of each stem was made. An arbitrary rating for disease severity was employed: no typical fruit-spots (0), slight spotting (1), medium spotting (2), heavy spotting (3). For each hand, one of these ratings was noted for spotting at the finger-tips and another for that on the inner sides of the inner whorl of fingers. It was considered unwise to establish a larger series of ratings, due to practical difficulties in carrying out assessments.

Fruit less than 20 days old was rarely more than slightly spotted, although it was often disfigured by the small lumps caused by thrips. Since thrips injury is readily distinguished from typical fruit-spot, there was little danger of confusing the two types of blemish. Slight-to-medium spotting was observed on 20- to 30-day-old fruit. In general, spots were distributed in a characteristic fashion on each finger, being particularly numerous on the inner sides near the finger-stalk, and also near the finger-tip on all sides. At this stage, the diameter of individual spots, including the water-soaked halo, rarely exceeded 1 mm. The intensity of spotting increased progressively with increasing age of fruit. At the time of harvest (80–90 days after 'shooting'), it was difficult to find a bunch completely free from spotting. Originally spot-free epidermis, in regions other than those mentioned above, may become spotted during later stages of maturity.

Table 6. *Relation of severity of fruit-spot to age of fruit*

Age of fruit (days from opening)	No. hands examined	Disease severity ratings (percentage of maximum possible)	
		Finger-tips	Inner sides
0–10	176	0	0
11–20	267	2·2	4·8
21–30	192	10·2	28·2
31–40	138	32·0	48·9
41–50	126	46·5	49·6
51–60	136	66·2	58·3
61–70	132	78·3	64·6
71–80	120	75·0	68·3
Over 80	157	84·2	61·4

It is important to note that the survey recorded in Table 6 was carried out after several weeks of very dry weather. Some further observations were made in the same plantations 1 week later, during which period there had been 2 days of relatively heavy rainfall (7·9 in.). It soon became evident that 0- to 10-day-old fruit was often quite heavily spotted, contrasting with the observations in the previous survey. Spots were restricted to the inner sides of the innermost whorl of fingers on each hand, and were more or less evenly distributed along each finger. Microscopic studies confirmed that *D. torulosa* was the causal organism. These findings provide further evidence that the presence of water is an important factor in relation to infection. The absence of spotting on young fruit examined in the earlier survey was probably due to unfavourable humidity conditions, limiting germination and subsequent infection.

Experimental infection. Laboratory experiments were set up to compare the susceptibility to infection by *D. torulosa* of fruits at different stages of maturity. Sections of peel measuring approximately 3·0 × 2·0 × 1·0 cm. were cut from newly harvested, washed bananas. One drop (diam. *c.* 0·75 cm.) of a tap-water conidial suspension was carefully pipetted on to the epidermal surface of each section. Control sections were inoculated with tap water only. The experiment was replicated ten times and the numbers of spots were counted after 24, 48 and 72 hr. incubation at 28–30° C. inside a damp chamber (Table 7).

The incidence of spotting on unopened fruit was significantly higher ($P < 0{\cdot}001$) than that on opened fruit. On the former, spots first became evident after only 9–12 hr. incubation; on fruit older than 10 days, spots did not become evident before 15–20 hr. There was a tendency for disease severity to decrease as maturity advanced. On all sections the numbers of spots increased up to about 72 hr. but, after this, there was little further increase. Spots on 20- to 60-day-old fruit were, in general, smaller than those on younger tissues.

This experiment was repeated several times, results comparable with those in Table 7 being obtained consistently. Sometimes the numbers of spots greatly exceeded those recorded above. In one experiment, mean counts of 26·5 and 19·7 spots were recorded on 60- and 80-day-old fruit, respectively.

Table 7. *Relation between age of fruit and incidence of fruit-spot caused by* Deightoniella torulosa

Mean no. spots per inoculum drop.

Time interval (hr.)	Age of fruit (days)						
	−2	1	10	20	30	60	80
24	62·2	11·0	2·3	0·5	0·4	0·4	0
48	> 100	16·6	4·4	2·8	2·5	0·6	0·6
72	> 100	22·0	6·6	5·2	4·5	1·9	3·4

No spots developed on control fruit.

Table 8. *Mean number spots per finger on variously aged fruit inoculated with* Deightoniella torulosa

Age of fruit (days)					
−1	10	20	30	70	100
392	183	122	125	76	78

Examination of spots on variously aged peel revealed certain interesting morphological and anatomical differences. On 1- and 2-day-old fruit, spots were prominently raised, rendering the peel rough to the touch. On older fruit, spots were similar to those developed under natural conditions, having a flat or slightly depressed, black centre. A transverse section of a typical raised spot is shown in Pl. 21, fig. 2. It is clear that the epidermis and several layers of underlying cells were caused to bulge, due to hypertrophy and radial elongation of certain relatively deep-seated mesophyll cells. There was no evidence that *D. torulosa* had penetrated as far as these mesophyll cells, suggesting that the response was evoked by substances diffusing in advance of invaded tissues. Histological details of a spot on older fruit are illustrated in Pl. 21, fig. 3, from which it is evident that there was no hypertrophic reaction.

Inoculations were also carried out in the plantations on fruits of different age. Each finger to be inoculated was examined carefully and a note was made of any spots that were present at the beginning of the experiment. Inoculations were performed by spraying fruit with a dense suspension of conidia in tap water. Fruit was then covered with air-tight plastic bags in order to retard evaporation of the droplets of suspension. Control fruit was sprayed with tap water only. The number of new spots developed on each finger was counted after 7 days, and the means from thirty replicates are given in Table 8.

As in laboratory experiments, unopened fruit was more severely affected than opened fruit, and the incidence of spotting decreased with advancing maturity. It is evident, however, that fruit ready for harvest was by no means completely resistant to infection. There was no tendency for spots to be concentrated in any particular area on each finger, contrasting with the distribution commonly observed under natural conditions.

Nature of resistance to infection. Although there was no evidence to suggest that fruit eventually becomes completely resistant to infection by *D. torulosa*, the results of the foregoing experiments certainly indicate that resistance increases with age of fruit. Sectioning showed that there was equally good germination on the variously aged fruit; also, appressoria were produced in similar numbers. On very young fruit a single epidermal cell, or a group of cells, immediately beneath the appressorium was usually necrosed, indicating that penetration had occurred. In contrast, cells on 70- to 100-day-old fruit were often apparently healthy, suggesting that the infection hypha, if developed, had not penetrated the epidermal wall.

It was observed that the combined thickness of the cuticle and epidermal cell-wall increases as the fruit matures. This thickening may act as a passive resistance to penetration, as in the case of certain other plant organs, for instance plums (Valleau, 1915), barberry leaves (Melander & Craigie, 1927) and tomatoes (Ainsworth, Oyler & Read, 1938). More detailed studies of this nature on banana fruit would be desirable.

The wax layer may indirectly enhance resistance to infection by making more difficult the adherence of the infection drop, especially in the case of very young fruit. As the fruit matures, it is more easily 'wetted'. Also, the orientation of young fruit (Text-fig. 1 B) results in water droplets running off the tips of the fingers under the influence of gravity. Only when the fingers have turned upwards do water drops rest for any considerable time on the inner sides. These factors may often result in young, susceptible fruit 'escaping' infection.

Distribution of fruit-spot on individual fingers

One possible explanation for the fact that spotting is often concentrated near the finger-tips, and also on the inner sides towards the finger-stalk, is that tissues in these regions are more susceptible than those elsewhere on the finger. An experiment was designed to test this hypothesis. Three hands were cut from a bunch of fruit that had 'shot' 10 days earlier. The individual fingers were removed and split longitudinally into two halves, outer and inner, respectively. After inoculating with a suspension of conidia of *D. torulosa*, the finger halves were incubated at 28–30° C. inside a damp chamber. For the purpose of recording, each finger half was divided into three equal lengths, denoted here as the finger-stalk, median and finger-tip segments, respectively. The number of spots developed on each segment was counted after 6 days incubation and the means are given in Table 9.

These data suggest that there were no marked differences between various regions of the finger in respect of susceptibility to infection. Thus the hypothesis presented earlier would appear to be invalid, and an alternative one must be sought and tested. Several observations during the course of investigation have led to a similar conclusion. Thus, when fruit was inoculated by spraying the complete bunch, the distribution of spots was more or less even along each finger.

Table 9. *Distribution of spots on banana fingers following inoculation with* Deightoniella torulosa

Hand	Whorl	Side of finger	Segment of finger		
			Finger-stalk	Median	Finger-tip
Proximal	Outer	Outer	24	28	34
		Inner	26	33	25
	Inner	Outer	19	23	24
		Inner	40	40	29
Intermediate	Outer	Outer	15	21	22
		Inner	31	27	28
	Inner	Outer	20	20	14
		Inner	26	26	11
Distal	Outer	Outer	32	31	30
		Inner	28	26	19
	Inner	Outer	22	15	20
		Inner	33	39	60

Seasonal occurrence of fruit-spot

The data illustrated in Text-fig. 2 show clearly the seasonal occurrence of fruit-spot on bananas exported to the United Kingdom. They were compiled from trade reports which describe qualitatively the general appearance of each shipment. An arbitrary series of ratings was applied to the commonly used descriptive terms: no spotting observed, negligible, –0; slight, trace, in evidence, occasional, –1; moderate, –2; heavy, widespread, severe, 'riddled', –3. A disease index for each month of 1957, 1958 and 1959, respectively, was calculated by summing individual shipment ratings and expressing the total as a percentage of the maximum possible score.

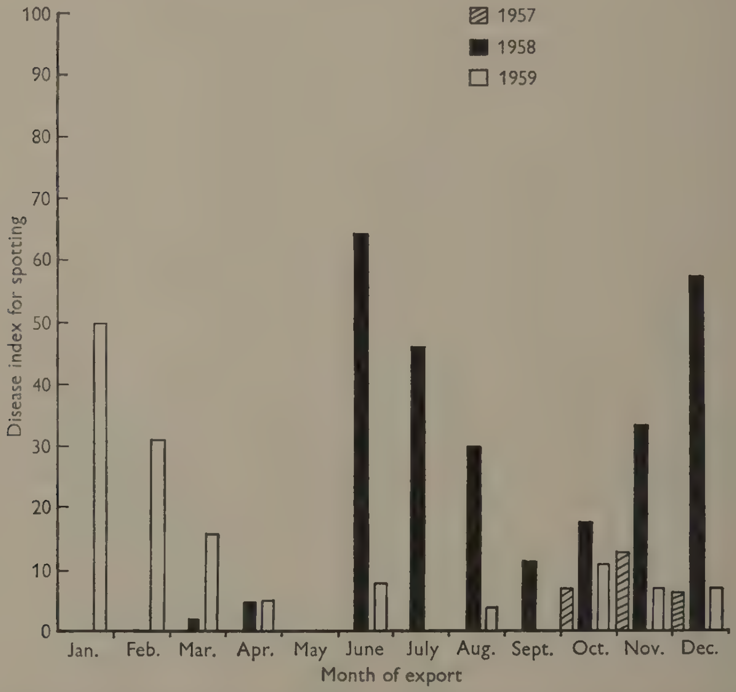
Since most severely spotted fruit is rejected in the tropics, these data cannot be considered to give a very accurate picture of seasonal trends. However, two major peaks of disease severity are evident, during the periods April–June and October–January, respectively. It is tempting to relate seasonal variation of spotting to the annual distribution of rainfall, for this too is usually heaviest during the same months.

There is evidence to suggest that the severity of 'speckling' varies considerably with locality. On the basis of climatic conditions, Jamaica can be divided into four major regions, namely western, central, northern and eastern. Each region has a characteristic distribution of rainfall (Leach, 1946). It would seem desirable to investigate in greater detail the relation between severity of disease and local climatic conditions.

INOCULUM SOURCES IN RELATION TO FRUIT-SPOT EPIDEMIOLOGY

Sporulation on dead, dying or diseased leaves

It has been noted by Meredith (1961) that, in Jamaican plantations, *D. torulosa* lives saprophytically on the collapsed dead or dying leaves which hang from the pseudostem. Here the fungus sporulates at all seasons. Such leaves are probably the largest source of conidia of the fungus and possibly they constitute the most important one in relation to fruit-spot epidemiology. It is known that conidia are readily liberated by the action of wind currents and rain, and are common members of the plantation air spora (Meredith, unpublished). Thus, although the bunch of fruit usually hangs above the collapsed leaves, it does not follow that conidia from this source are not available for infection, provided that other conditions are favourable. It may be that very heavy rainfall would tend to wash many conidia downwards and away from the fruit.



Text-fig. 2. Seasonal occurrence of fruit-spot on Jamaican bananas exported to the United Kingdom in 1957, 1958 and 1959.

Intense spotting has been observed on the uppermost sides of wind-blown bunches that had been lying for 2–3 days on the plantation floor in rainy weather (Meredith, 1961). This was almost certainly due to large numbers of conidia being washed on to the fallen fruit from overhanging dead leaves. A similar intensity of spotting was also observed on unfallen fruit on which a leaf had collapsed and died.

D. torulosa sporulates abundantly on leaves that it has infected to produce 'black-spot' disease (Ashby, 1913; Stahel, 1934). Although this disease is not of great economic importance in most Jamaican plantations, one severely attacked plot in the Christiana area (2400 ft.) was visited. The youngest leaves of most plants were heavily spotted, while many older leaves displayed large areas of marginal browning. A detailed examination of every bunch of fruit in the plot revealed an exceptionally high incidence and severity of spotting. Even the youngest fruit examined, 2 days after opening, was slightly spotted. On fruit of all ages, spotting was most intense on the inner sides of the innermost whorl of fingers in each hand. This outbreak of fruit-spot was probably related to the presence of an abundant inoculum source in the form of 'black-spot' diseased leaves. On almost every plant, one or more diseased leaves were situated above the bunch of fruit, so that conidia liberated therefrom would tend to impact on the inner sides of the fingers underneath. The accumulation of rain and dew drops on the inner sides would favour the occurrence of infection there.

Old leaf-spot lesions, caused by *Mycosphaerella musicola* and invaded saprophytically by *D. torulosa*, possibly form a further source of incoulum. It would be of interest to discover whether the severity of fruit-spot is correlated with that of Mycosphaerella leaf-spot.

Sporulation on the perianth and other flower parts

After opening, the female flowers, consisting of perianth, style and staminodes, slowly wither and decay, and finally absciss. This leaves at the tip of the finger a callused scar which persists as a characteristic feature of the mature fruit. During decay of the flower parts many fungi, e.g. species of *Fusarium*, *Acrothecium*, *Cephalosporium*, *Nigrospora*, *Pleospora*, and *Gloeosporium musarum*, can be observed sporulating thereon. It was of particular interest to find that *D. torulosa* also sporulates in this region.

On 'Lacatan' bananas, withering of the perianth and staminodes usually commences 10–20 days after opening; the style remains healthy for a longer period, but this also slowly decays from the stigma end. Flower parts from fruits of various ages were removed and shaken thoroughly in water in order to dislodge spores. Drops of the resultant turbid suspension were examined microscopically and the abundance of viable conidia of *D. torulosa* was recorded. (Table 10).

Sporulation of *D. torulosa* on the decaying flower parts suggested a possible explanation for the characteristic finger-tip spotting frequently observed on naturally infected fruit. Rain and dew droplets falling on to the flower parts may, before evaporating, run for some distance down the finger. If this water is spore-laden, conidia will settle on the epidermis. In this way a disease gradient will be established on each finger, being most severe close to the inoculum source, that is, the flower parts. Surface sections of fruit collected in the early morning after heavy dew deposition revealed such a gradient in numbers of conidia of *D. torulosa* and other fungi. Early stages of infection by the former fungus were evident.

An experiment was set up to determine whether the removal of the flower parts, before they begin to decay, had any effect on the severity of fruit-spot. The healthy perianths, staminodes and styles were removed from every hand on each of twenty 10-day-old bunches; the unwithered overhanging transition leaves were also removed. A further twenty bunches of comparable age were selected but not treated in any way. The intensity of spotting at the finger-tips and on the inner sides was recorded 6 weeks later, during which period there was heavy rainfall. It was quite clear that de-flowering had no appreciable effect in reducing finger-tip spotting. Both on treated and on untreated fruit, the over-all incidence of spotting was exceptionally high. Thus, the explanation for tip-spotting suggested above appears to be invalid. An alternative and more likely one is discussed below.

Table 10. *Sporulation of* Deightoniella torulosa *on decaying flower parts*

Age of fruit (days)	Condition of flower parts	Conidia observed
0–10	Healthy, undecayed	None
11–20	Decay commencing	Very few
21–40	Decayed and withered	Several
41–60	Decayed and withered	Several
61–80	Perianth often dehisced, style usually persistent	Very few

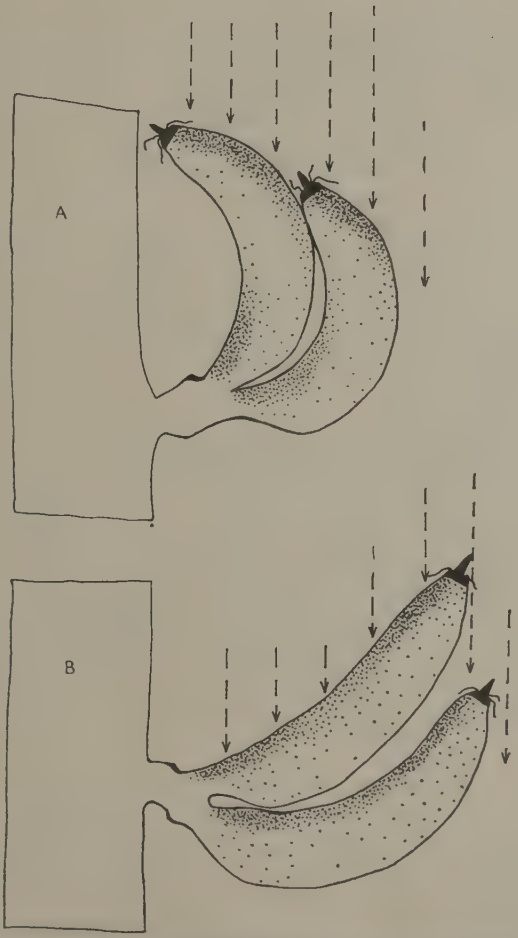
Sporulation on transition leaves and bracts

The last leaf to be produced before 'shooting', referred to by Summerville (1944) as the 'spade leaf', usually withers while the bunch matures. This is true also for the bract-like leaves which are borne on the main stalk proximal to the large, uppermost hand. There is good evidence that these structures are a source of infection, for *D. torulosa* sporulates abundantly on them. After liberation, conidia would tend to move downwards, particularly in water drops, and impact on the fruit below. Sometimes there is a direct transfer of conidia; there was often very severe spotting on areas of fruit peel in contact with decaying bracts.

The spathe-like bracts which subtend each hand usually absciss and fall away from the fruit before decay commences. Because of this, it seems unlikely that they are of great importance as an inoculum source, even though *D. torulosa* may sporulate on them. Rarely the bract may become trapped within the bunch and this is sometimes associated with a localized dense spotting on nearby fingers.

Sources of inoculum: discussion and conclusions

It seems probable that the major sources of inoculum available for fruit infection vary with local plantation conditions. In a well maintained plantation where dead and dying leaves are removed shortly after their collapse, the decaying flower parts, transition leaves and bracts will be of some importance. It has been observed repeatedly that the incidence of fruit-spot is highest in poorly maintained plantations and lowest in clean ones. During the rainy seasons it is possible to find the deep olive-green masses of conidiophores and conidia of *D. torulosa* on almost every dead leaf petiole. It is strongly recommended that plantation hygiene be maintained at the highest level practicable, particularly when heavy rainfall is expected. In this way some reduction in fruit-spot may be effected. Intense fruit-spot may be expected if the foliage leaves are severely affected by 'black-spot' disease.



Text-fig. 3. Diagrams showing various degrees of curvature of banana fingers as observed under natural conditions. The distribution of fruit spot caused by *D. torulosa* is indicated by dots. Arrows represent paths of movement of falling conidia.

The results of inoculation and de-flowering experiments failed to explain satisfactorily the distribution of spots on individual fingers. It has been noticed repeatedly that on young fruit, where the inner sides are sloping downwards, almost horizontal or sloping upwards slightly, spotting is restricted to these sides. This is suggestive of the downward movement of conidia, in air or in water drops, resulting in impaction on these sides. Data in Table 6 show that up to 30–40 days after 'shooting', finger-tip spotting is markedly less than that on the inner sides. As the finger curves upwards, so the intensity of spotting towards the finger-stalk on the inner sides increases. This could be due to conidia being washed down the finger towards the cushion. Finger-tip spotting develops soon after the finger has assumed an almost vertical position. It was found that a consistent relation exists between the distribution of spots on the outer and inner sides near the finger-tip and the degree of curvature of the finger. When the finger-tip was bent over towards the main stalk, spotting was heaviest on the outer sides (Text-fig. 3A); in such cases, there was often very little spotting on the inner sides. When the finger-tip pointed outwards, spotting was usually concentrated on the inner sides (Text-fig. 3B). Downward-moving conidia will tend to impact chiefly on the areas of peel indicated by arrows in Text-fig. 3; this may be the most important factor governing the distribution of spotting.

Although de-flowering had little effect on the development of tip spotting, it is almost certain that a small proportion of spots are caused by conidia developed on the perianth or style.

Recent studies have shown that *D. torulosa* is a common member of the plantation air-spora (Meredith, unpublished). The broad foliage leaves may trap some of these spores. The confluence of leaves at the crown of the pseudostem constitutes a funnel which would tend to direct rain-water, possibly spore-laden, on to the bunch of fruit below. It was noted by Meredith (1961) that the large proximal hand is usually more severely spotted than lower hands. Presumably this hand traps many of the downward falling conidia, thus reducing the amount of inoculum which reaches lower hands.

An unusual form of fruit-spot

Several instances of a severe and unusual form of spotting were observed during the course of investigation. One plantation in the Christiana district was particularly noteworthy, but isolated cases were also found on the lowland plains of St Catherine. The largest spots observed were approximately 6 mm. diam., raised in a convex fashion and dark-brown or black in colour (Pl. 21, fig. 4); they are best described as being 'scab-like'. Similar smaller spots, 1–3 mm. diam., usually had a water-soaked halo and closely resembled typical 'speckle'. On most affected fingers, there was a continuous series of spots ranging from typical 'speckle' to the large, scab-like spots. On fruit up to 25 days old, spotting was restricted to the inner sides of the fingers. On older fruit, the outer sides were often affected also, but to a less extent. Occasionally, there was characteristic finger-tip spotting similar to that described earlier.

On isolation plates inoculated with pieces of scab-like tissue, *D. torulosa* appeared in a high percentage of cases. An attempt was made to induce the development of these spots by experimental inoculation with *D. torulosa*. Apparently healthy 10-day-old fruit was collected from the Christiana plot and, after washing, was sprayed with a dense suspension of conidia. Many small 'speckles' developed after 48 hr. incubation at 28–30° C. After 5–7 days, some of these spots had developed into characteristic scab-like lesions. These observations suggest that *D. torulosa* is the cause of scab-like spots which develop under natural conditions. It may be that the physiological condition of fruit peel tissues affects symptom expression. The suggestion that peculiar soil conditions are responsible for this is now under investigation.

OTHER BANANA DISEASES CAUSED BY *DEIGHTONIELLA TORULOSA*

On leaves

A 'black-spot' disease of leaves of 'Gros-Michel' bananas in Jamaica was first described by Ashby (1913), and the causal fungus was given the name *Cercospora musarum* Ashby (syn. *D. torulosa*). An early stage of disease is indicated by black pin-point spots on the main veins near the margin of the lamina. Each spot increases in size, eventually becoming lenticular and surrounded by a bright yellow border. Further increase in size occurs in a fan-like fashion towards the edge of the lamina. Individual spots may unite and give rise to a broad zone of pale-brown, dry tissue around the periphery of the lamina. Ashby (1913) and Ogilvie (1928) isolated the fungus from small black spots, but the studies of Stahel (1934) provided convincing proof of the pathogenicity of *D. torulosa*. Meredith (1961) found that the details of germination and penetration of banana fruit, leading to the development of fruit-spot, are closely similar to those on leaves, as described by Stahel (1934). 'Black-spot' can be observed on 'Lacatan' bananas in most Jamaican plantations. However, with the exception of isolated plots at Christiana and elsewhere, it is not of great economic importance.

Small-scale inoculation experiments were designed to test the ability of isolates of *D. torulosa*, obtained from fruit-spots, to infect banana leaves. A suspension of conidia in tap water was sprayed into the narrow funnel of a very young, unfurled leaf—the 'heart-leaf' (Leach, 1946). After inoculation, the open end of the funnel was closed by means of a rubber band: six leaves were inoculated in this way. Controls were provided by similar leaves sprayed with tap water only. After 2½ days, small, dark-brown spots, sometimes up to 1 mm. diam., appeared on inoculated leaves; each spot was surrounded by a pale yellowish green halo. After 4 days, the spots had elongated in the direction of the main veins; further development occurred in the manner described above. In all cases spotting was densest on the 'right side' of the leaf which bordered the inside of the funnel (according to Leach (1946), the 'left side' of the leaf is that half which unfurls first in the 'heart-leaf' stage and, in the fully open condition, is on the left-hand side of the leaf when viewed from the pseudostem). Small black spots also appeared in the cavity of the midrib and it seems likely, as pointed out by Stahel (1934), that similar spots observed under natural conditions are also caused by *D. torulosa.* There was no development of spots on control leaves.

Details of leaf infection by *D. torulosa* were observed on material that was inoculated and incubated at 28–30° C. inside moist chambers. Germination and penetration occurred in the manner described by Stahel (1934). From these various observations it was concluded that isolates of *D. torulosa* which cause fruit-spot may, under favourable conditions, also cause 'black-spot' of leaves. Similarly, it was found that 'black-spot' isolates infected banana fruits and caused typical 'speckling'.

On fruit

In Bermuda, Ogilvie (1924, 1925, 1927, 1928) described a disease ('black-tip') of 'Cavendish' banana fruits caused by *D. torulosa.* The disease is characterized by a black discoloration of the skin at the finger-tip. This blackening is usually on one side of the fruit only and is bordered by a narrow grey or light-yellow margin. The complete finger is seldom affected and ripening appears to inhibit further development of the disease; mycelium of *D. torulosa* may develop on the surface of the diseased tissues. The fungus is localized in the outer peel tissues, the amount of internal decay produced being negligible. Wardlaw (1932) observed isolated cases of disease on 'Cavendish' and 'Lacatan' bananas in Trinidad. He considered that infection originates in the perianth or style, and that penetration of hyphae into the fruit takes place as a result of delayed or incomplete formation of the corky layer which normally seals the end of the fruit after decay of the floral parts. A direct connexion between hyphae in the base of the style and those in the tissues of the fruit skin was observed.

'Black-tip' is uncommon in Jamaica and so far in these investigations only a few examples have been found. In every case, fruit affected by this disease was also heavily spotted. The plot at Christiana was of particular interest in that there was severe fruit-spot, 'black-tip' and 'black-spot', all caused by *D. torulosa.*

Inoculations were designed to induce 'black-tip' experimentally. A young bunch of 'Lacatan' bananas (approximately 15 days from the time of 'shooting') was sprayed with a dense suspension of conidia of *D. torulosa* until run-off occurred. Conidia were obtained from the same culture that was used for leaf-inoculation. The bunch was then covered with a plastic bag which was sealed at both ends. After 7 days, the fruit was very severely spotted and two adjacent fingers on the large proximal hand displayed characteristic 'black-tip' symptoms (Pl. 21, fig. 5). Many similar inoculations have been carried out during the course of this investigation, chiefly with the object of inducing fruit-spot. In many instances, 'black-tip' developed on heavily spotted fingers but not on those that were only slightly spotted.

In other inoculations, a small incision was made in the corky tissue at the tip of the finger; conidia were applied to the wound. In all instances, typical 'black-tip' symptoms developed after 7 days incubation at 28–30° C. in a saturated atmosphere.

These experiments demonstrate that isolates of *D. torulosa* from fruit-spot are able to cause 'black-tip'. The converse is also true, for isolates of *D. torulosa* obtained from naturally occurring 'black-tip' infections caused typical fruit-spots after inoculation.

Discussion

Investigations reported in this paper have emphasized the importance of external factors, especially rainfall and humidity, in relation to infection of banana fruits by *D. torulosa*. Not only is liquid water essential for a high incidence of germination and infection, but it also appears to be an important agency bringing about dispersal of the fungus. It is understandable, therefore, why spotting is most severe during or following periods of heavy rainfall. Whether rainfall has any indirect effect on disease severity, through its effect on the growing plant, has not been determined. The importance of rain was further indicated by observations in which severity of fruit-spot was related to age of fruit. At first, since laboratory work demonstrated the high susceptibility of very young fruit, it was difficult to explain why similarly aged fruit in the field was rarely affected. In further surveys, made during rainy periods, the converse was true. The absence of spotting on earlier-examined young fruit was probably a result of unfavourable humidity conditions, for there is no reason to believe that inoculum was unavailable.

The work of Johnston (1932) and Johnston & Slocum (1932) on a spotting of Gros Michel bananas growing in Central and South America may be relevant to the present investigation. The fruit-spot which they described (often referred to within the trade as the 'Johnston fruit-spot') appears to differ from typical 'speckle' in certain morphological respects. These workers concluded that spotting is a physiological disorder, brought about by unfavourable climatic conditions, namely heavy rainfall and high humidity. However, they reported that fungal mycelium was observed in some sections of spotted tissue. It would be of interest to discover whether inoculation with *D. torulosa* of Gros Michel bananas growing under different soil conditions results in the development of 'Johnston fruit-spot'. In this paper it is noted that the morphology of fruit-spot varies according to local conditions, possibly due to soil factors affecting the physiological reaction of infected fruit tissue.

My thanks are due to Mr N. A. Davidson and Miss V. E. Green for technical assistance. This work is published by permission of the Banana Board, Kingston, Jamaica, W.I.

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EXPLANATION OF PLATE 21

Fig. 1. Germinated conidium of *Deightoniella torulosa*, with three germ-tubes.

Fig. 2. Vertical section through fruit-spot on very young, unopened banana; hypertrophy and radial elongation of deep-seated parenchyma cells.

Fig. 3. Vertical section through fruit-spot on 60-day-old banana.

Fig. 4. Scab-like spots caused by *D. torulosa.*

Fig. 5. 'Black-tip' of bananas caused by *D. torulosa.*

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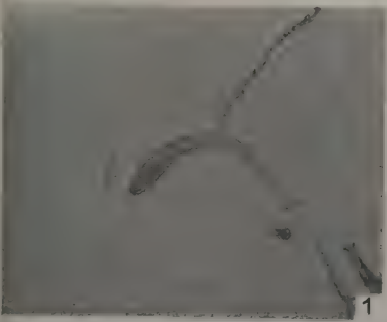

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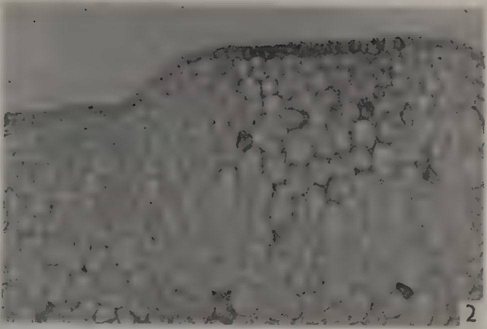

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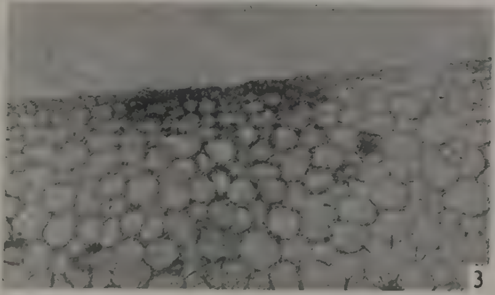

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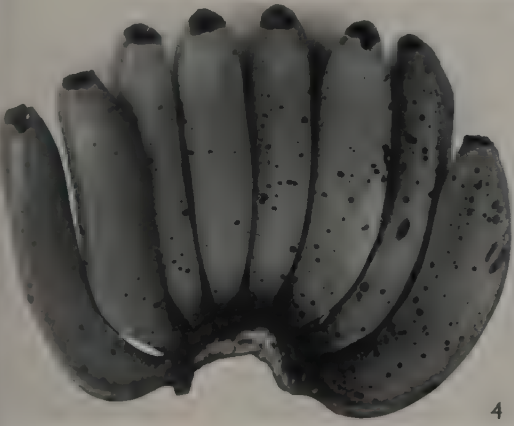

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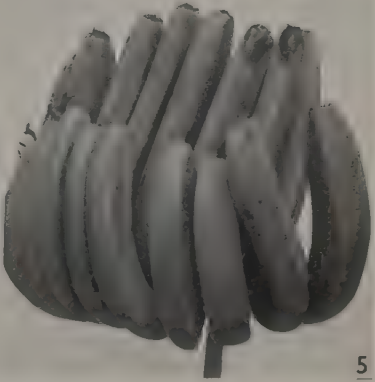

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MORPHOGENESIS OF MYCELIAL STRANDS IN THE CULTIVATED MUSHROOM, *AGARICUS BISPORUS*

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(With 1 Text-figure)

When growing from an inoculum providing a suitable food base over an inhospitable surface, such as that of glass, or through a non-nutrient medium, such as unsterilized soil, the mycelium of *Agaricus bisporus* becomes organized into mycelial strands. The morphogenesis of the strands appears very similar to that previously observed in strand development by *Merulius lacrymans*, *Phymatotrichum omnivorum* and *Agaricus campestris*. A number of adjacent and parallel-running leading hyphae growing out from a food base into the surrounding soil become bound together and ensheathed by a profuse development of their finer branches (tendril hyphae), which are thin-walled and aseptate. Strand development is acropetal.

By the use of sterilized, acid-washed quartz sand as a non-nutrient medium surrounding the food base, it could be demonstrated that strand development amongst the outgrowing mycelium was inversely correlated with the concentration of free nutrients in the medium. At very low nutrient concentrations, mycelial growth was sparse but strand development was conspicuous; at higher nutrient concentrations, the reverse occurred.

Mycelial strands superficially resemble rhizomorphs, but Garrett (1956, 1960) has proposed that the term 'rhizomorph' be restricted to those fungal organs possessing an apical meristem, like the rhizomorph of *Armillaria mellea*. Mycelial strands, on the other hand, possess no apical meristem but are gradually built up around one or more leading hyphae, by a process of acropetal development. The best-known type of mycelial strand is that of *Merulius lacrymans*, causing dry rot of timber; its morphogenesis was originally analysed by Falck (1912) and has been the subject of recent work by Butler (1957, 1958). These two workers have shown that, when the mycelium of *M. lacrymans* is travelling over or through a non-nutrient medium such as brickwork, concrete or soil, the branch hyphae do not fan out in all directions as on an agar plate but wrap themselves around one or more (adjacent) leading hyphae, forming a sheath-like cortex. A similar type of morphogenesis has been demonstrated for *Phymatotrichum omnivorum*, causing Texas root rot of cotton and other crops, by Rogers & Wakins (1938). A second and rather distinct type of strand morphogenesis has been described for *Helicobasidium purpureum*, causing violet root rot of various crops, by Valder (1958). Mycelial strands of *H. purpureum* are built up by the coming together of a number of leading hyphae growing out into soil or other non-nutrient medium from a food base. Coherence is attained by the interweaving growth of the leading hyphae, by short side-branches of limited growth that often terminate in a hook, and by frequent anastomoses. Mature strands of these two types present a distinctive appearance under a hand-lens, inasmuch as those of the first type (e.g. *Merulius lacrymans*) have a smooth and well-finished appearance, whereas those of *Helicobasidium purpureum* appear more roughly and coarsely woven.

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Observations by Hein (1930) on strand formation by *Psalliota campestris* (this binomial is a synonym of *Agaricus campestris*; see Dennis, Orton & Hora, 1960) suggest that the morphogenesis is essentially of the *Merulius lacrymans* type, as described by Falck (1912) and Butler (1958). The object of the investigation to be described in this paper was to confirm and amplify Hein's observations, and also to confirm experimentally the general observation that mycelial strands are formed only in media having a low content of free nutrients (Garrett, 1960). As *Agaricus bisporus* is more easily procured at short notice than is *A. campestris*, the former species was chosen for this investigation. The behaviour of strands of *A. bisporus* in compost colonization has been studied with special reference to their inoculum potential by Garrett (1954), but he made no observations on strand morphogenesis.

STRAND MORPHOGENESIS

The isolate of *A. bisporus* employed in this investigation was freshly obtained from a packet of pure-culture commercial mushroom spawn, and the stock culture was maintained on 4 % malt-extract agar. The method of observing the course of strand development was adapted from that devised by Valder (1958) for *Helicobasidium purpureum*. Petri dishes (9 cm. diam.) were filled with 50 g. air-dried garden soil, which was then adjusted to a moisture content of 60 % m.h.c. After 24 hr. to permit equilibration of the soil moisture, a shallow depression was made in the soil in the centre of each dish, to receive the inoculum disk. Inoculum disks of 10 mm. diam. were cut out with a cork borer 10 mm. behind the growing margin of a colony of *A. bisporus*, 3–4 weeks old on 4 % malt-extract agar (20 ml. agar/9 cm. Petri dish), and one was placed, mycelial surface upwards, in the central soil depression previously made in each dish. A large rectangular cover-glass was then pressed down over the inoculum disk and surrounding soil. The Petri dishes were incubated inside polythene bags at 22·5° C., and microscopical observations on strand development were made in situ at regular intervals, by direct illumination through the cover-glass. These observations were supplemented by others made on mycelium growing out from an inoculum disk on to a glass slide within a moist chamber. Drawings illustrating early stages in strand formation were made under the camera lucida (Fig. 1).

Under these conditions, macroscopically visible strands became apparent near the inoculum disk within 3–4 days. With further development, some strands of 200–300 μ diam. were seen, but most of them did not exceed 80 μ. By the time that outwards mycelial growth from the inoculum disk had ceased, few individual hyphae were seen in the soil, except near the food base, where they persisted for longer. Older strands teased apart in 3 % potassium hydroxide were found to consist of a central core of more

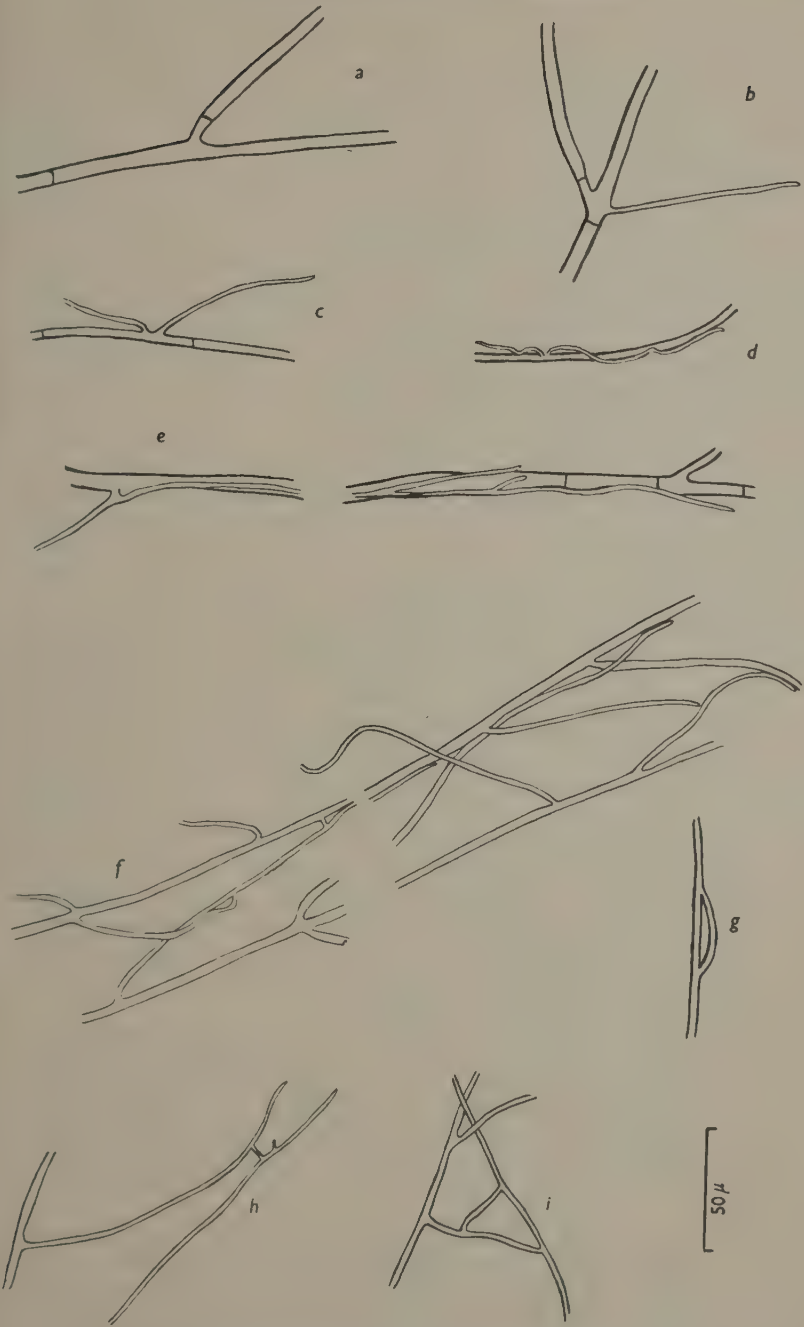

Fig. 1. *Agaricus bisporus*. Early stages in formation of mycelial strands. For explanation see text.

or less parallel, septate hyphae of 3–7 μ diam., surrounded by a cortex of much narrower (1–2 μ diam.) and more irregularly running hyphae, as described and figured by Hein (1930) for the strands of *Agaricus campestris*. A hyphal covering of needle-shaped crystals, as reported by Hein, was also occasionally noted.

Strand formation was found to proceed somewhat as follows. Mycelial growth outwards from the food base (i.e. the inoculum disk) was initiated by a number of robust leading hyphae, which branched at fairly wide intervals to form progressively thinner branches (Fig. 1 *a*, *f*). Most of the primary and larger secondary branches of these leading hyphae grew away from their parent hyphae, but a minority—especially where leading hyphae were crowded together—formed a more acute angle and followed the parent hypha. Other branches, at first growing away from the parent hypha, were observed to change direction and grow alongside a larger hypha that they chanced to encounter (Fig. 1 *f*). Fresh hyphae growing out from the food base, after the leading hyphae had developed, tended to be of smaller diameter and made only a limited outwards growth. Some of these anastomosed at their tips with one of the leading hyphae, or followed it in further growth. Others branched frequently and anastomosed amongst themselves to form a network near the food base (Fig. 1 *i*).

The second stage in strand development was characterized by the development of numerous fine, thin-walled, aseptate hyphae as branches from the older regions of the main hyphae, and their branches (Fig. 1 *b*, opposite a primary branch, and *c*). These hyphae will be distinguished as 'tendril hyphae', following the nomenclature of Falck (1912) and Butler (1958) for *Merulius lacrymans*. These tendril hyphae tended to follow closely the growth of a larger hypha, to which they became appressed (Fig. 1 *d*, *e*); growth could be either forwards or backwards along the large hypha, as described by Butler (1958) for *Merulius lacrymans*. The original tendril hyphae in turn branched frequently to form yet finer tendrils, which grew around the larger hyphae, filling up interstices in the developing strands and ensheathing them. Tips of some originally wide branch hyphae were seen to narrow abruptly, and then to grow in the manner of tendril hyphae. Various types of hyphal anastomosis (Fig. 1 *f*–*i*) also helped to consolidate the developing major strands, which increased in thickness with accretion of minor strands.

Strand branching developed when some of the tendril hyphae grew away from the main strand to follow a wide-angled, stout branch hypha, or an unrelated wide hypha that had happened to cross the path of the strand.

From these various observations, therefore, it can be concluded that strand morphogenesis in *Agaricus bisporus* is very similar to that already described for *A. campestris*, *Merulius lacrymans*, and *Phymatotrichum omnivorum*.

STRAND FORMATION IN RELATION TO NUTRIENT STATUS OF THE GROWTH MEDIUM

When growing on 4 % malt-extract agar, *Agaricus bisporus* covers a 9 cm. plate in about 3 weeks with a dense growth of aerial mycelium; mycelial strands are not formed except occasionally near the point of inoculation in old colonies. Sometimes, however, good stranding has been observed on an area of the plate over which the layer of agar has been shallow, on account of an inequality in the bottom of the Petri dish. A series of Petri dishes and slant tubes, each containing 20 ml. agar, was inoculated with *A. bisporus* to test the effect of nutrient concentration on strand formation. The following agars were employed: water agar, soil-extract agar (500 g. soil shaken in 1000 ml. water), 0·5 and 4 % malt-extract agars. On 4 % malt-extract agar, dense mycelium but few strands were produced; on 0·5 % malt-extract agar, total mycelial growth was much reduced but stranding was appreciably increased. Both on water and on soil-extract agar, the fungus made only a sparse growth of appressed mycelium, but conspicuous strands were formed. Strand formation was generally better on the agar slants than on the plates, especially in the lower part of the slant tube where condensed water persisted for longest on the glass wall. Both Hein (1930) and Styer (1930) reported that strand formation by *A. campestris* developed best under conditions of high moisture.

As even purified agar contains a residuum of fungal nutrients, it was decided to use acid-washed quartz sand for a further study of the effect of nutrients on strand formation. The sand was dry-sterilized in the Petri dishes at 155–160° C. for 3 hr., and sterile water or nutrient solution was added later. Inoculum disks were set up in the dishes of sterile sand as described above for dishes of unsterilized soil, but the cover-glasses had circles of 2 cm. diam. marked on them, so as to provide a ring transect for counting mycelial strands around the inoculum disk, as employed by Butler (1957) in her study of *Merulius lacrymans*.

Experiment 1. Sterilized acid-washed sand was compared as a non-nutrient medium for mycelial growth and strand formation from a food base with sterilized and unsterilized unwashed sand and with unsterilized garden soil, all at a moisture content of 60 % m.h.c. Five replicate dishes were set up in each series, and a count of all strands of 40μ or more in diameter, crossing the ring transect around each inoculum disk, was made at intervals up to 21 days (Table 1).

Table 1. *Strand formation in various non-nutrient media*

	Mean no. strands formed after			
	4 days	7 days	14 days	21 days
Soil, unsterilized	2·6	11·6	11·4	*
Sand, unwashed, unsterilized	0	0	1·6	2·8
Sand, unwashed, sterilized	0	0	8·0	9·2
Sand, acid-washed, sterilized	0	0	3·0	4·2

* After 21 days in unsterilized soil, strands had begun to disintegrate.

The best and most rapid strand formation occurred in unsterilized soil, and the next best in sterilized unwashed sand.

Experiment 2. Sterilized acid-washed sand was employed for this experiment in conjunction with a complete nutrient solution (N) and with a series of dilutions therefrom. The nutrient solution was made up as follows: $NaNO_3$, 1·2 g.; KH_2PO_4, 1·0 g.; $MgSO_4.7H_2O$, 0·5 g.; glucose, 20 g.; distilled water, 1000 ml. Four replicate dishes of sterile sand were brought to a moisture content of 60 % m.h.c. with the undiluted nutrient solution and with each of the following dilutions made from it: 1/5, 1/25, 1/100 and 1/200 N. As most of the strands measured only 20–40 μ diam., all strands crossing the ring transect were counted under the stereoscopic dissecting microscope at 21 days after inoculation (Table 2).

Table 2. *Strand formation over a range of nutrient concentrations in sterilized sand*

	Mean no. strands
Sand + water only	40·5
Sand + medium N	1·0
Sand + 1/5 N	5·2
Sand + 1/25 N	16·0
Sand + 1/100 N	27·7
Sand + 1/200 N	27·0

The results of this experiment show clearly that the frequency of strand formation in the mycelium growing outwards from a food base can be inversely correlated with the concentration of free nutrients in the surrounding medium.

I am deeply indebted to Dr S. D. Garrett for valuable advice and criticism during the course of this work.

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SOME FACTORS INFLUENCING SPORANGIUM FORMATION OF A *PHYTOPHTHORA* SPECIES ISOLATED FROM LUCERNE IN CERTAIN SALT SOLUTIONS

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AND

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A *Phytophthora* species from lucerne, possibly *P. megasperma*, formed sporangia readily when disks of V-8 juice agar with mycelium were incubated in a shaken inorganic nutrient solution for 24 hr. at room temperature. Adding ethylenediamine tetra-acetic acid stimulated sporulation markedly on V-8 juice $CaCO_3$ agar (pH 6·5) but only slightly on V-8 juice NaOH agar. Tap water, concentrated to one-fifth of its original volume by boiling, and aqueous soil extracts stimulated sporulation on V-8 juice NaOH agar. Sporulation was slight in tap or double-distilled water. Copper in the inorganic salt solution at concentrations as low as 0·2 p.p.m. Cu^{2+} inhibited formation of sporangia, and at 0·1 p.p.m. induced formation of abnormal types.

The production of sporangia in artificial media by a *Phytophthora* isolate, causing a root disease of lucerne in the United States (Erwin, 1954), has been erratic and infrequent (Erwin & Kennedy, 1957). More information concerning factors influencing sporangial formation of *Phytophthora* spp. which sporulate sparsely may contribute towards a better understanding of their ecology in soil and eventually to knowledge of the mechanisms involved in sporulation.

Although some *Phytophthora* spp. sporulate when either mycelium or agar disks of mycelium are transferred to Petri's (Tucker, 1931), Wills's (1954), and M/100 KNO_3 (Gooding & Lucas, 1959; Leonian, 1925; Tucker, 1931) solutions, from pea broth to distilled water (Leonian, 1925), or when colonized organic material (including lucerne stems) is transferred to running water (Klotz, 1940), none of these methods induced consistent sporulation of the isolate from lucerne.

Bingham & Zentmyer (1954) reported that abundant sporulation of *P. cinnamomi* Rands occurred when colonized lucerne stems were placed in an aerated nutrient solution. In our study the lucerne *Phytophthora* also sporulated well when lucerne stems or agar disks and mycelium were placed in a modified Hoagland's solution (Hoagland & Arnon, 1950). The influence on sporulation of an inorganic salt solution and the sporulation-reducing effect of copper will be reported here. Part of this study was previously reported in a brief abstract (Kennedy & Erwin, 1960).

METHODS

Agar (15 g./l.) media were prepared by either boiling or autoclaving 200 g. of each organic material for 20 min., filtering, and adding distilled water to make 1 l. Reaction of V-8 juice agar (200 ml. of Campbell's V-8 juice per l.) was adjusted to pH 6·5 with $CaCO_3$ (V-8 $CaCO_3$) (Miller, 1955), NaOH (V-8 NaOH), or $Ca(OH)_2$ (V-8 $Ca(OH)_2$).

The complete inorganic nutrient solution (pH 5) was a modification of that described by Hoagland & Arnon (1950) and was prepared as follows: (A) macrosalts: KH_2PO_4, 0·001 M; KNO_3, 0·005 M; $Ca(NO_3)_2.4H_2O$, 0·005 M; $MgSO_4.7H_2O$, 0·002 M; (B) microsalts: 1 ml. per l. of the following stock solution: H_3BO_4, 2·86 g.; $MnCl_2.4H_2O$, 1·81 g.; $ZnSO_4.7H_2O$, 0·22 g.; $CuSO_4.5H_2O$, 0·08 g.; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0·2 g.; and distilled H_2O to 1 l.; (C) chelated iron according to Jacobsen (1951): 1 ml./l. of the following stock solution: ethylenediamine tetra-acetic acid (EDTA) 26·1 g. ($8{\cdot}9 \times 10^{-5}$M), KOH 15 g., $FeSO_4.7H_2O$ 24·9 g., and distilled H_2O to 1 l. The chelated iron solution will be referred to as FeKEDTA and the potassium salt of ethylenediamine tetra-acetic acid as KEDTA. Solutions were made with distilled water, redistilled in a glass still. In all tests, except where otherwise designated, the solutions were not sterilized.

To each 250 ml. wide-mouth Erlenmeyer flask (washed with sulphuric acid-potassium dichromate cleaning solution), 100 ml. of the solution to be tested was added. Each flask was inoculated with 5 agar disks of mycelium to minimize the possible effects of the agar medium. The disks were obtained from the outer margins of Petri dish cultures, 10–20 days old. Disk size varied among tests from 4 to 10 mm. diam. but was constant for any one test. Solutions were continuously agitated on a laboratory shaker which made about 110 excursions per minute. Laboratory temperatures ranged from 21 to 26° C. After 22–24 hr. numbers of sporangia in a microscope field 1 mm. diam. were estimated and recorded in the following six classes: none (0), 1–3 (trace), 3–10 (1), 11–20 (2), 21–30 (3), and above 30 (4). Numbers of sporangia were recorded for at least three loci on each disk. Each test included two to four replications and was repeated several times. The *Phytophthora* culture previously identified as *P. cryptogea* Pethyb. & Laff. (Erwin, 1954) was from a single hyphal tip isolated from a diseased lucerne root. The authors acknowledge with appreciation the recent assistance of Miss Grace Waterhouse, Commonwealth Mycological Institute, Kew, who pointed out that paragynous as well as amphigynous antheridia were formed by this fungus. According to present taxonomic concepts (Waterhouse, Grace M., 1954, Key to the species of Phytophthora recorded in the British Isles, *Com. Mycol. Inst. Myc. Papers*, no. 57), this finding would necessitate designating the lucerne fungus as a small oospore form of *P. megasperma* Drechs. Since at the time this paper was ready for press only limited comparative studies of the morphology of the two fungi had been made, confirmation of the identity of the lucerne fungus will be given in a later paper.

RESULTS

Effects of the inorganic nutrient solution on sporulation

In preliminary tests the complete nutrient solution was inoculated with disks of agar and mycelium from each of several agar media: V-8 $CaCO_3$, corn meal, oatmeal, garbanzo bean, lucerne seed, lucerne tap root (1-year-old plants), and potato, the latter either with or without dextrose (20 g./l.). On the plate, no sporulation occurred after 10–20 days' growth on any of the media. In the nutrient solution, sporangia were produced (values of 2–4) on all media except potato dextrose, corn meal, and oatmeal agars; but in glass-distilled or tap water, the fungus sporulated sparsely (values of 0–1).

Since sporangia were produced on potato agar but not on potato-dextrose agar, the effect of dextrose (20 g./l.) was tested on both potato and V-8 $CaCO_3$ agars. Sporulation was much greater on disks of V-8 $CaCO_3$ agar (value of 3) and on potato agar (values of 2–3) than on either medium including dextrose (trace to 1).

Since growth and sporulation were consistently greatest on inoculum grown on V-8 $CaCO_3$ agar, this medium was used for subsequent experiments. Although sporangia were produced on the mycelium from a V-8 $CaCO_3$ broth medium, sporulation was not uniform enough for a quantitative evaluation.

Agitating solutions by means of a shaker greatly increased both the numbers of sporangia and the rate at which they were formed. In a typical test a sporulation value of 3 was recorded in the agitated but only 1 in the non-agitated solution. It was assumed that agitation improved aeration. Sporulation values of 2–3 in both sterilized and non-sterilized solutions were obtained in a series of five tests. Varying the pH from 4·7 to 8 with NaOH or HCl likewise had no appreciable effect, though pH 4 greatly reduced sporulation.

In one test excellent sporulation of *P. citrophthora* (R. E. & E. H. Smith) Leonian, *P. parasitica* Dastur, *P. drechsleri* Tuck., and *P. cinnamomi* was obtained in the inorganic solution as against almost none in tap water. *P. cinnamomi* sporulated only after 48 hr. incubation, however.

Effect of potassium ethylenediamine tetra-acetate on sporulation

To determine what groups of compounds in the complete nutrient solution contributed to the sporulation process, the macrosalts solution alone, as well as in combination with FeKEDTA, KEDTA, and the microsalts solution, were inoculated with the fungus on V-8 $CaCO_3$ disks. Sporulation occurred only in the macrosalts solution which contained FeKEDTA or KEDTA. Iron, as sulphate or tartrate, added to the macrosalts solution had no effect on sporulation. It appeared that the effect on sporulation was due to KEDTA in combination with the macrosalts.

The increase in sporulation due to KEDTA, however, was much less striking on V-8 NaOH agar disks than on V-8 $CaCO_3$ agar (Table 1).

Since EDTA is capable of forming stable complexes with calcium as well as other metals (Martell & Calvin, 1952) and is considered inert biologically (Hutner, Provasoli, Schatz & Haskins, 1950), the effect on sporulation appeared likely to be associated with the removal of calcium from V-8 $CaCO_3$ agar.

Table 1. *Comparison of sporulation on V*-8 NaOH *agar with that on V*-8 $CaCO_3$ *agar in two inorganic solutions and in double-distilled water*

	Agar medium	
	V-8 $CaCO_3$	V-8 NaOH
	Sporulation value	
Solution		
Macrosalts	Trace	3–4
Macrosalts + KEDTA	2	4
Double-distilled water	Trace	Trace

Although in this and subsequent tests a higher sporulation value was consistently obtained on V-8 NaOH than on V-8 $CaCO_3$ agar, the sporangia were smaller. Mycelium on V-8 NaOH agar was appressed and radial growth reduced (in 10 days at 27° C. colony diam. on V-8 $CaCO_3$ was about 90 mm. and on V-8 NaOH only 45 mm.). The increase in growth on V-8 $CaCO_3$ agar appeared to be due to the presence of calcium since V-8 $Ca(OH)_2$ agar supported a type and rate of growth similar to V-8 $CaCO_3$ agar.

The small increase in sporulation due to the presence of KEDTA in the macrosalts solution on V-8 NaOH agar was discernible in most tests but not in all. The slight response may be due to a metal contamination inactivated by KEDTA in the V-8 NaOH medium. Miller (1956) reported that V-8 juice contained 0·4 p.p.m. of copper.

Effect of concentrated tap water and soil extracts on sporulation

Wills (1954) showed that sporulation of *P. parasitica* var. *nicotianae* Tuck. was enhanced by concentrating tap water to 1/100 of its original volume by boiling. Only a trace of sporulation on V-8 NaOH agar occurred in tap water (pH 7·4). However, when tap water was concentrated to one-fifth of its original volume by boiling (pH 9·2), an increase in sporulation to a value of 3–4 occurred, but in water boiled to one-tenth of its volume (pH 9·3), sporulation was reduced to a trace. The effect of boiling appeared to be due to the increase in concentration of salts in the boiled water.

In several tests in which agar disks of mycelium were placed in wet soil, abundant sporulation (approx. 3) was usually evident within 24 hr. on soils of different types. Soil extracts (pH varied from 7·4 to 8·4) were prepared by filtering 300 g. air-dried soil in 1000 ml. distilled water. These extracts induced abundant sporulation (value of 3) but distilled water did not.

Effect of copper on sporulation in the macrosalts solution in presence and absence of KEDTA

During an earlier study it was noted that a certain source of distilled water contained a contamination of 1 to 2 p.p.m. of free copper (information from the Department of Soils and Plant Nutrition, University of California, Riverside). Although growth on various agar media was not inhibited by this amount of copper, no sporulation occurred in inorganic salt solutions prepared with this source of distilled water. However, when KEDTA ($8{\cdot}9 \times 10^{-5}$ M) was included in the solution normal sporulation occurred.

Several tests were conducted to study the effect of low concentrations of copper in solution on sporulation. Copper was supplied at concentrations of 0–1 p.p.m. copper by addition of $CuSO_4.5H_2O$ to the macrosalts solution in the presence, as well as in the absence, of KEDTA. In this test complete inhibition of sporulation occurred at 0·2 p.p.m. copper and above in the macrosalts solution, but normal sporulation occurred in the solution when KEDTA was present (Table 2). In another test reduction of sporulation occurred at 0·1 p.p.m. copper in the absence of KEDTA but sporulation was normal in the presence of KEDTA. In solutions containing small amounts of free copper sporangia became abnormally elongated and frequently ruptured.

Table 2. *The effect of copper in the macrosalts solution on sporulation in presence and absence of KEDTA*

	Copper (p.p.m.) in macrosalts solution			
	0·0	0·2	0·4	1·0
	Sporulation value			
Without KEDTA	3	0	0	0
+KEDTA	4	3–4	3	3

Discussion

Wills (1954), in an excellent, comprehensive study of the effect of various factors on sporulation, showed that certain carbonate, nitrate, phosphate, or chloride salts added to a dilute solution of KCl, KH_2PO_4, and $MgSO_4.7H_2O$ increased sporulation of *P. parasitica* var. *nicotianae*. Carbonates were particularly effective. He was not able, however, to separate the effects of pH from that of balance of salt components on sporulation. Gooding & Lucas (1959), working with the same fungus, showed that the greatest number of sporangia occurred at pH 6 in a 0·01 M-KH_2PO_4–K_2HPO_4 buffer solution. Since the lucerne fungus did not sporulate in Wills's solution, it appears that species of *Phytophthora* differ in their requirements for sporulation.

Although sporulation was augmented by the balanced inorganic macrosalts solution and slightly stimulated further by the effect of the metal chelating compound KEDTA, not enough data are yet available from our study to draw conclusions concerning a chemical cause for sporulation, other than that sporulation appears to be increased by use of certain inorganic salt solutions, soil extracts, and concentrated tap water. Since it is difficult to determine a chemical effect when an organic medium such as V-8 juice agar is employed, future work to study sporulation on a synthetic medium has been started. Schmitthenner (1959) has reported that *P. cryptogea* sporulated on plates of either dilute synthetic or natural agar media. It may be possible that the inorganic salt solution in our studies exerted a simple dilution effect on the nutrients in the agar disk; however, it is difficult to explain why dilution with distilled water failed to stimulate sporulation.

The inhibition of sporulation at 0·2 p.p.m. Cu and the appearance of atypical sporangia in a concentration of copper as low as 0·1 p.p.m. indicate the sensitivity of the fungus to copper ion. Klotz (1940) reported inhibition of zoospore germination of *P. citrophthora* with 3·3 p.p.m. of Cu^{2+}. Zentmyer & Marshall (1959) reported prevention of sporangial formation of *P. cinnamomi* in soil extract at concentrations of 1 p.p.m. or greater of copper.

Microbiological approaches to the use of EDTA in research have been reviewed by Hutner *et al.* (1950) and Reischer (1951). These workers indicated that EDTA appeared to be biologically inert and capable of tightly binding heavy metals. DeKock & Mitchell (1957) showed that chlorosis and stunting of mustard caused by the presence of divalent metallic ions such as Ni and Cu in culture solution did not occur when EDTA was present as the chelate either of iron or of the toxic metal. Reischer (1951) used EDTA as a non-toxic metal carrier for studies of microelement nutrition of certain members of the Saprolegniaceae in synthetic media. The removal of the inhibitory effect of copper from solution by KEDTA in our work lends further evidence that this metal chelating agent might be a useful tool for study of inorganic nutrition of *Phytophthora* spp.

Since V-8 juice, according to Miller (1956), contains 0·4 p.p.m. of copper, the slight increase in sporulation on V-8 NaOH inoculum in the inorganic nutrient solution (Table 2) associated with KEDTA may have been due to the chelation of this metal. The reason for the stimulating effect of KEDTA in the nutrient solution inoculated with V-8 $CaCO_3$ agar disks of mycelium is difficult to interpret since calcium is not toxic but necessary for optimum growth of several *Phytophthora* species (Davies, 1959; Lopatecki & Newton, 1956) as well as in our studies with the lucerne *Phytophthora* (to be reported in a later paper).

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PROCEEDINGS, 1961

ANNUAL GENERAL MEETING 1960

Saturday, 3 December 1960. The sixty-fourth Annual General Meeting was held in the rooms of the Linnean Society of London at 2.0 p.m. with the President, Mr E. C. Large, in the Chair.

After the Minutes of the previous Annual General Meeting had been read and signed, the President recorded with regret the deaths during the year of Dr M. Wilson, a former President; of Dr W. Watson, an Honorary Member, and of Mr W. D. Buckley. He then briefly reviewed the year's activities.

The Treasurer presented his Annual Statement which was accepted *nem. con.* on the proposition of Mr W. C. Moore, who referred to the exceptional services which the retiring Treasurer had rendered to the Society. The President then made a presentation to Mr Buddin on behalf of the Society.

The following Officers, members of the Council and members of Committees were elected for 1961: *President*: Miss G. M. Waterhouse; *Vice-President*: W. Buddin; *Treasurer*: S. A. Hutchinson; *Secretary*: J. G. Manners; *Foray Secretary*: Mrs N. Montgomery; *Editors*: S. D. Garrett and Miss G. M. Waterhouse; *Members of Council*: P. R. Day, F. C. Murray, Mrs P. E. Perrott (retiring members, J. L. Harley, J. C. Hopkins, J. Webster); *Members of Foray Committee*: Miss M. P. English, Miss M. Holden, F. B. Hora, B. E. Plunkett, R. W. Rayner; *Members of Plant Pathology Committee*: C. Booth and R. L. Lucas.

The President then moved that Mr E. W. Mason be elected an Honorary Member of the Society; this motion was carried with acclamation.

Dr P. W. Brian, Vice-President, then took the Chair, and the President gave his address, entitled 'Pursuits of Mycology'. A vote of thanks to the President for his address was moved by Dr P. H. Gregory and carried with acclamation.

MEETINGS

5 January 1961. Meeting held at Birkbeck College, University of London, at 10.45 a.m. After the formal business, the Chairman of the Plant Pathology Committee, Dr F. Joan Moore, took the Chair, and there followed a Symposium organized by the Plant Pathology Committee.

THE LEAF AND ITS MICROFLORA

A. H. Bunting (Reading). Introduction.

P. H. Gregory (Rothamsted). The leaf as a spore trap.

Microbes colonize leaves either by continuous spread, as from a bud scale or seed coat, or by discontinuous spread, which involves the arrival of a disseminule through air in the form of dust or in a water droplet. Spore-trapping by the leaf requires efficiency of collection and retention. Aerodynamic factors affect collection by sedimentation and impaction. In wind-tunnel experiments, in general, leaves collect more efficiently at low wind speeds, but impaction efficiency increases as wind speed increases. Margins, narrow bases, tips, and projections, collect more by impaction than do wider parts of the blade; part of the unequal distribution of lesions on leaf surfaces may be accounted for by local differences in trapping efficiency. Few dry spores are deposited on the under sides of leaves. Elongated spores tend to land with characteristic orientation. Some air-borne spores carry electrostatic charges and may possibly be attracted to plant surfaces bearing unlike charge. A leaf can collect spores in splash droplets (put into the air by impact of a falling rain- or drip-drop with a liquid film). By this process even very small spores can be collected efficiently on both upper and lower leaf surfaces; even without aid from wind a leaf can receive spores from impacts occurring 20 cm. or more below it. Retention efficiency of a leaf is increased by surface roughness, but the importance of the hydrophobic or hydrophilic nature of surfaces on spore retention, like several other topics mentioned above, merits further study.

E. Evans (Chesterford Park). Relationship between the physical characteristics of a banana leaf and the distribution of leaf spot lesions (*Mycosphaerella musicola* Leach).

Brunskill (1956) demonstrated that water drops would bounce from the surface of certain crop plants, such as peas. The biological significance of this phenomenon to the study of herbicides has since been described by Dewey (1956). However, no one appears to have examined the significance of this phenomenon to plant pathology.

Banana leaves show bouncing characteristics similar to those of pea leaves, although not all banana leaves nor all parts of a given leaf illustrate this phenomenon. Bouncing does not occur from the surface of veins, although it takes place quite freely from the interveinal areas. The result is that water drops of 1000 μ in diameter applied to the leaf tend to congregate on the veins, although smaller drops of approximately 300 μ in diameter tend to have a more random distribution over the leaf.

Spores of *Alternaria solani* present in the applied water drops naturally tend to accumulate on the veins in large drops but their distribution in small drops is again almost at random.

Leach (1946) described two basic symptom patterns of the leaf spot disease of bananas, namely 'tip-spotting' and vertical 'line spotting' symptoms. A closer examination of either of these patterns shows that they are composed of short lateral lines of lesions with a tendency to follow the veins. A count of the number of lesions present on and off the veins in two samples of naturally infected (Lacatan variety) banana leaves shows that approximately 60 % of the lesions are present on the veins, despite the fact that the veins only occupy approximately 10–20 % of the total leaf area.

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J. T. Martin (Long Ashton). The leaf cuticle in relation to fungal invasion.

An account was given of work on the leaf cuticle as a mechanical barrier to invasion and on the occurrence in the cuticle of substances affecting the germination of spores. The chemistry of the cuticle was reviewed and a method was described by which the cuticle components were determined quantitatively and which yielded wax and cutin fractions for chemical examination and bioassay. Analytical values were reported showing the wide differences in the cuticles of leaves of different species.

Comparisons of the cuticles of susceptible and resistant leaves of lime, apple, rose, turnip and Euonymus failed to reveal differences in the deposition of waxy materials and cutin that might account for resistance. Infection of leaves by powdery mildew or scab seemed to stimulate the production of waxy material and led to loss of cutin; a virus infection had no effect. Under the conditions of the test used, some waxy fractions stimulated and others inhibited the germination of spores; the strongest inhibitory effect was shown by cutin acids obtained from the cutin. The influence of these findings, of the changing nature of the surface layers and of other materials in cuticles upon the resistance of leaves to infection was discussed.

F. T. Last (Rothamsted). The saprophytic flora of the phyllosphere.

The environment on and near roots is recognized as a zone of intense microbial activity. Similarly, leaves, perhaps through plant secretions, also provide a substrate for a flora whose functions seem to be as diverse as that on roots. Already numerous examples have been found of leaf-inhabiting micro-organisms fixing nitrogen while others have been shown (*a*) to secrete materials which stimulate rooting, and (*b*) to be antagonistic to other micro-organisms.

The saprophytic flora of the phyllosphere consists predominantly of pigmented and non-filamentous micro-organisms which rarely occur as frequently in other habitats, e.g. *Pseudomonas trifolii*, *Flavobacterium* spp., *Chromobacterium* spp., *Rhodotorula* spp. and *Sporobolomyces* spp.

These organisms are each found on a wide range of plant hosts but their numbers vary greatly with (*a*) the season, (*b*) the development of the host and (*c*) the incidence of plant parasites. The ballistospores of *Sporobolomyces* spp. are often found in large numbers in humid atmospheres, but little is known of the possible dispersal mechanisms of the other saprophytes. The movement of some, however, from the surfaces of germinating seeds to seedling leaves has been demonstrated.

D. H. Lapwood (Rothamsted). Varietal differences in the susceptibility of potato leaves to blight.

Leaves of different potato varieties differing in susceptibility modified the behaviour of a leaf pathogen, *Phytophthora infestans*, either at an early stage in the infection cycle, before or soon after penetration of the host, or later during the invasion of the leaf tissues, Particular reference was made to a series of laboratory tests in which the behaviour of the fungus on leaves of a wide range of varieties differing in susceptibility and maturity was compared with that on variety Majestic. The tests assessed the ease of infection of leaf and leaf axil, the sporing capacity and rate of advance within leaf tissue. The degree of resistance or susceptibility shown by leaves of some of the varieties in these tests, was related to, and agreed well with, the field performance of varieties in blight epidemics, as assessed from the infection and destruction of marked leaves within crops.

Leaf susceptibility differed within a variety as well as between varieties, varying with leaf age and nutritional status; varieties were made more susceptible by hastening maturity, e.g. by shortening the day length.

A. E. Flood (East Malling). The biochemistry of the leaf in relation to disease.

This paper is concerned with events taking place when a pathogen has penetrated the leaf cuticle of the host. Discussion of such events must take into account the shifting balance between two metabolic systems existing side by side. In the leaf, new metabolic pathways may arise or existing ones assume greater or less predominance. Thus a different type of carbohydrate metabolism may appear and the products of this metabolism may be channeled at least in part through a route other than the tricarboxylic acid cycle. The regulation of the rate of respiration by processes of oxidative phosphorylation is also commonly disturbed and in addition there is the possibility of the system of electron transport from substrate to the ultimate acceptor, oxygen, being altered. At the same time the importance of any changes in the hormonal balance cannot be ignored.

Similarly, the metabolism of the pathogen is influenced or disturbed by the changes mentioned above. Recent work has indicated the great diversity in metabolism of even one type of compound by different species and strains of micro-organisms. The host-pathogen relationship must therefore be viewed as a complex to be studied as the whole dynamic equilibrium.

R. K. S. Wood (Imperial College) opened the general discussion.

REVIEWS

Plant Pathology. An Advanced Treatise. Vol. II. *The Pathogen.* Edited by J. G. Horsfall and A. E. Dimond. (New York and London: Academic Press, 1960). Pp. xiv + 715, illustrated. Price: $22.00.

As stated in the Preface, this volume deals with 'the antecedents of disease, how disease originates, in short what causes disease'. This is certainly true of most of the book but it is not obvious how chapters on fungicides and nematocides fall within that programme. Clearly, however, chemical (and physical) control methods must receive substantial treatment in a treatise of this character and it is a matter of little consequence where they are placed. The fifteen chapters of the text are self-contained, so that it is open to the reader to group them in any order that suits his purpose, as will be done here.

'Reproduction of bacteria, actinomycetes and fungi' gives a synoptic view of sexual and asexual modes of reproduction throughout these groups. Special attention is given to the former, both in morphological and physiological aspects, because of their importance for evolutionary theory and for the major taxonomic divisions. Also, they are fundamental to those later developments which have so notably extended the fields of microbiology, genetics and plant pathology.

By and large the older mycologists carried their classifications down to the species level. But later and more intensive work, mainly carried out under controlled conditions and with finer techniques, has shown that many of the so-called species actually consist of a cluster of subspecies (biotypes, physiological races, etc.), some of which are static while others are liable to vary. How these arise and how far they can be subjected to genetical analysis is set out in two chapters: 'Heterokaryosis, saltation and adaptation' and 'Genetics of pathogenicity'.

Both of these topics are large and intricate: the first, which is the newer, is in process of active development and still has many obscure or controversial features; the second, which has arrived at a state of comparative stability, embraces such a formidable mass of well-authenticated detail that the highest skill in arrangement and condensation is called for if the reader is to escape bewilderment. In spite of these difficulties, both chapters are admirably clear, even to a reader whose genetical experience is limited.

A special point may be made regarding the author of 'Genetics of pathogenicity'. He appears to be one of the two persons in the three volumes of the treatise who have submitted their completed texts to experts in particular branches of their subjects. Whether this led to any material alteration, in the form of addition or subtraction, is not stated—quite possibly not. The procedure is a good one and could well be imitated.

Four chapters deal with physiological processes operative in the causation of disease: these are 'Spore germination', 'Mechanical ability to breach the host barriers', 'Chemical ability to breach the host barriers' and 'Toxins'. The first two of these relate to fungi, the others to fungi and bacteria.

As spore germination is a necessary preliminary to parasitic invasion, an account of it seems natural enough. Besides, there is an immense accumulation of data on the subject, though most of them have been obtained incidentally in work that had another objective. There are some physiological problems that are special to germination, such as the nature of dormancy and the processes involved in the transition from the comparative rest of the ungerminated spore to the full-speed growth of the mycelium. But practically nothing is known about either of these. Inevitably, therefore, the chapter is limited to the listing and grouping of largely empirical findings, with little of general principle emerging. It possesses considerable value, however, as a guide to the practical worker.

The chapter on mechanical penetration of host barriers contains much speculation, as the author himself observes. It is not always easy to come to grips with some of the views expressed, largely because they are so wrapped up and qualified that it is far from clear just what is being said. A surprising view is that structures such as lignitubers, callosities, etc. (as described and figured in chapter 2 of vol. I) are not due to fresh depositing of cell-wall material but to the swelling, through enzymic action, of pectic substances already present. There is nothing in the known action of pectolytic enzymes that would lead to a swelling of their substrates, i.e. if by 'swelling' is meant an effect comparable with what happens when a piece of dry gelatine, say, is placed in water. A curious section is the one headed 'Barriers due to the absorption of the pathogen's enzyme system'. This refers to work done in the reviewer's laboratory, and the surprising thing is to see it described in a chapter on the *mechanical* breaching of barriers.

The author of 'Chemical ability to breach host barriers' also strays at times from his title. He describes a variety of barriers to invasion, among them the cuticular barrier, even though he agrees that the available evidence strongly favours breaching by physical means. Another barrier to invasion is an unfavourable pH of a particular tissue and this could be overcome by any fungus able to alter the pH to a more favourable level. This is quite feasible. But one would think that a barrier due to 'absence of essential nutrients' would be unbreachable by any means, in the absence of artificial adjustment. Treatment of enzymic breakdown of cell walls is limited to about a quarter of the text and is less thorough than that given in chapter 5 of vol. 1, both in the range of effects described and in the choice of citations.

There are many points arising in this chapter and in its opposite number in vol. 1 which the writer of this review would like to have taken up if space had allowed. One general point will be made and this concerns the replacement of the old 'protopectinase'—which was nothing more than a name of convenience for an enzyme system that destroyed the wall structure of parenchymatous cells—by a group of enzymes which, though still fluctuating, bring about fairly well defined reactions in vitro. This replacement is undoubtedly a marked step forward and one that will in due course give precision to the various behaviours of different parasites. Nevertheless, many of the features to which much attention is now being given are probably of greater interest enzymologically than plant pathologically. The key event as regards attack on living tissue is in the breakdown of solid structural components of the plant, with loss of coherence of the tissue and more or less concomitant death of the protoplasts, and the plant pathological effect is much the same whether subsequent action of the enzyme system degrades to ultimate molecules or stops at some intermediate stage. There are, it should be noted, no real grounds for thinking that the products of cell-wall breakdown are of critical importance to the parasite, which by that time has gained free access to the more varied contents of the dead protoplasts. There seems to be a danger, then, that an over-emphasis may be placed on 'after-the-battle' events, to the neglect of the more important problems that arise at an earlier stage of the process: such as, for example, the inhibition of maceration (and killing) by physical or chemical action of the host tissue, and the fundamental problem of the relation of macerating to killing action, as yet insufficiently explored. The significant material for this kind of work will still be standard pieces of living tissue, as heretofore.

Substances harmful to living cells can often be isolated from fungal or bacterial cultures. Hence the extensive and controversial literature which is assembled in the chapter on toxins. Hence also the necessity for the Dimond and Waggoner 'rules of proof'. Perhaps these rules do not go far enough. A toxic substance may undoubtedly be formed in a lesion, but by that time the tissue may already have been killed by another toxin. There could therefore be a primary toxin which was responsible for the initiation of attack, and, as the lesion grew, other toxins could arise which would modify and probably intensify the rate of attack. Wherever there is breakdown of cell walls there is, on the evidence available, a nearly simultaneous killing of the protoplasts by a colloidal substance, almost certainly an enzyme (if not pectolytic, then possibly proteolytic or lipolytic) and this would be the toxin primarily concerned. Even the well-authenticated toxin of *Pseudomonas tabaci* is a case in point, for it will be noted that the related organism, *Ps. angulata*, which does not possess the specific toxin, nevertheless produces a necrotic lesion.

Two chapters on somewhat generalized lines are included in this volume. The title 'Interaction of pathogen, soil, other microorganisms in the soil, and host' can be interpreted very widely and the authors have done so, with the result that the text spreads into the topics of at least ten of the chapters of the treatise. The argument is sometimes difficult to follow, not so much because reactions occurring in soil are notoriously complex, but because it tends to be overloaded with detail, not all of which is relevant to the point under discussion. An interesting feature is that, despite the title of the treatise, there is a short account of the mycorrhizal phenomenon. The reviewer considers that a whole chapter, setting out what is known of the physiology of endotrophic mycorrhizae, would be a desirable addition to the treatise. There are many points of contact between pathogenicity and symbiosis, the one shading into the other according to the strain of the microorganism used or to the circumstances prevailing. The fact that the outcomes may be so very different does not preclude the likelihood that the fundamental processes for both have a great deal in common, so that the study of the one may well throw light on that of the other.

The chapter on 'The nature, origin and evolution of parasitism' is, according to the Preface, the only one assigned to parasitism, all the others being concerned with pathogenicity. Parasitism, it should be added, had already been defined as 'the ability of one organism to procure food from another', as against its customary use as the equivalent of pathogenicity. One is therefore surprised to find words which connote pathogenicity occurring much oftener than the word 'parasitism' in the table of contents of this chapter. If the reader follows the accepted convention, he will find the phrase 'good pathogens but relatively poor parasites' rather startling. On the new and narrower definition of parasites it should mean effective destroyers of living material but not effective in procuring food, which does not seem to make sense. It means, in fact, destructive pathogens, although unspecialized in their pathogenicity. Throughout the chapter it is tacitly assumed that pathogenic specialization rests on a requirement for special food substances. One need think only of the wilt-producing *Fusaria*, with their high degree of pathogenic specialization combined with their catholic food requirements, to realize how baseless this assumption is. Even if it were true, the precise requirements of a highly specialized pathogen in vivo are so utterly unknown that the only meaningful interpretation of specialized parasite (in the narrow sense) is to equate it with specialized pathogen, which is the customary usage. On this view the distinction drawn by the Editors loses its force, and the contents of this chapter could very suitably have been distributed among the other chapters of the book. Many of its statements, it should be added, are highly debatable.

There are two chapters on viruses, 'The multiplication of viruses' and 'Virus inactivation in vitro and in vivo'. The centre of interest in the former is the nature and structure of the virus particle, as revealed by the electron microscope, by X-ray diffraction, by centrifugation and by chemical analysis, or deducible from the results of inoculation experiments. The second has certain practical aspects, but here again much of fundamental interest can be derived from a study of the effects of chemical and physical treatments on virus activity. Both authors are at pains to stress the inherent difficulties in work of this kind—thus, 'it is easier to get results than to interpret them' in the first named and 'there are many pitfalls for the unwary' in the other—so that knowledge in this fascinating and widely appealing subject is still very fragmentary. The ordinary reader will probably be more struck by the amount of knowledge that has accrued and by the ingenuity with which this was achieved. He will perhaps be disappointed at finding no hint in either chapter of how the very large virus particle, even after it has shed its protein sheath, is able to pass through the outer wall and ectoplast of the epidermal cell, or how it manages to kill the host cell so quickly when it invades certain kinds of unsuitable hosts. Incidentally, one wonders how much this hypersensitive reaction has in common with what looks to be a very similar phenomenon in fungal parasitism.

The two chapters on fungicides are important components of this volume. 'Fungicidal chemistry' describes briefly the ways in which chemical substances can be damaging to fungal cells and follows up with a detailed consideration of the mode of action of various chemicals, inorganic but more especially the newer organic, which are in practical use. 'Fungitoxicity' is complementary to the preceding, giving a full discussion of the parts (e.g. cell membrane, nuclear spindle) and activities (e.g. enzyme reactions) that are affected by fungicides. The general reader, though he may, like the reviewer, be insufficiently equipped to follow some of the discussions of molecular structure, will welcome these chapters for the general picture presented, and the specialist worker will also benefit by having the large mass of literature assembled and critically reviewed.

The final chapter, 'Nematocides', deals concisely with nematodes as agents of plant disease: their varied life-histories, whether ecto- or endo-parasitic, cyst- or non-cyst-forming; their occurrence, whether on foliage or seed or in soil. This leads up to practical control methods by the use of fumigants and of modern machinery. This will be a most useful chapter to the mycological plant pathologist who is liable, at any time, to have his problem complicated by the presence of nematodes, and all readers will probably enjoy the experience of getting down to the plain facts of a practical job, as a restful change from the 'ivory-tower' discussions which crop up repeatedly in many of the other chapters.

W. BROWN

Aquatic Phycomycetes. Second revised and enlarged edition. By FREDERICK K. SPARROW, Jr. (Ann Arbor, U.S.A.: University of Michigan Press, 1960). Pp. xxv + 1187, 8 plates, 91 text-figures. Price: $22.50.

In his review of the first edition of this book (these *Transactions*, **26**, 174, 1943) Dr B. Barnes stated that all students of aquatic life would find *Aquatic Phycomycetes* of very great help. This has indeed proved true, and those who knew that Prof. Sparrow was preparing a new edition must have awaited it eagerly and with impatience because so much new work has been done in this field since 1943.

The text is greatly enlarged, but even the additional 400 pp. scarcely reflect the fact that over 50 genera (two of which are new), not treated in the first edition, are now included. Notable among these are *Physoderma* (aquatic spp. only), *Coelomomyces*, *Sorosphaera* and other Plasmodiophorales on aquatic plants, all the Saprolegniaceae, *Pythium* (with a key based on Middleton's monograph), *Phytophthora* (four spp. only), and *Ancylistes*. The inclusion of descriptions of the many taxa newly described since 1943 (up to 1956) will be of great service to those without ready access to the original papers.

Hand in hand with the enlargement has gone a thorough revision of the text and a reconsideration of the classification in the light of modern knowledge. For example, *Catenaria* and *Coelomomyces* are now included in the Blastocladiales and the Hypochytriaceae is raised to ordinal rank.

This time, the printing, which is excellent, has been done in the Netherlands, presumably to keep down the cost, but even so many will wince at the price of $22.50. But the book is undoubtedly essential for anyone working on water moulds. Dr Barnes's suggestion in his review that the second edition should be divided into two volumes has passed unheeded. There is no doubt that the work would be far more handy in that form than the present tome (weight $5\frac{1}{2}$ lb.). Next time one volume for the Chytridiales and another for the remainder will be essential, especially if the Zoopagaceae are included, which would seem desirable if the book is to live up to its title.

G. M. WATERHOUSE

Monografia dos Fungos Micropeltaceae. By A. CHAVES BATISTA (Publicacao No. 56, Instituto de Micologia, Universidade do Recife, 1959). Pp. 519, 228 figures.

The Micropeltaceae or 'fly-speck' fungi are treated by Prof. Batista as one of seven families in the Microthyriales. He divides them into four subfamilies based on the structure of the thyriothecium. Within these, forty-five genera, sixteen of them new, are distinguished according to the following pattern: shape of thyriothecium, simple or multiple hymenium, circular or stellar ostiole against irregular dehiscence; spore colour and septation; and the presence or absence of paraphyses. Approximately 450 species are included, about half of them described as new by the author and his colleagues.

This catalogue will henceforth be a 'set book' for all students of these fungi who will have to make critical decisions on the merits of the many taxonomic proposals therein.

C. BOOTH

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